CHROMATOGRAPHIC STUDIES ON PRESERVATIVES IN THE WOOD OF SOME CONIFERS, ESPECIALLY OF THE GENUS *ABIES*, *PICEA* AND *PINUS*

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The chemical composition of woods varies from genus to genus and there are also differences between the species of any one genus. The composition of a chloroform extract of a wood differs from that of a bark or another part of a plant. The phenols in the wood are of great interest as they represent the constituents of lignified tissue, and also because they are biochemically important compounds, participating in various oxidation–reduction reactions. Coniferous woods frequently contain large quantities of other chemical compounds, particularly resins, which have a complex chemical composition, and also terpenoid materials. The presence of these compounds in conifers depends both qualitatively and quantitatively on the development of balsam ducts[2].

Investigations were made to establish whether the presence of these naturally occurring phenols and resin components in the woods of various *Abies*, *Picea* and *Pinus* species would interfere with the chromatographic detection of different constituents of wood preservatives in treated timber.

The preservatives studied were pentachlorophenol, benzene hexachloride, *o*-phenylphenol, tributyltin oxide and polychloronaphthalene. Attempts were made to find a general reagent which would detect all of these compounds, the methods described previously being more specific. WETZEL *et al.*[7] described the use of 4,4′-bis-(dimethylamino)triphenylmethane for the detection of pentachlorophenol, which gives a green colour after treatment with the leuco-base. An alternative reagent for the detection of this compound was a copper–pyridine reagent[6]. For the detection of tin-containing preservatives, for example tributyltin oxide, the dithizone method has been described[1]. The general detection of phenolic constituents of wood was described by HANOVER AND HOFF[4].

Initially, detection of these wood preservatives in the following eight commercial coniferous woods, *Araucaria angustifolia*, *Juniperus communis*, *Larix decidua*, *Pinus palustris* and *P. sylvestris*, *Pseudotsuga* sp., *Tsuga heterophylla*, *Taxus* sp. and one deciduous wood *Quercus* sp. was studied. Later investigations were made on a number of woods collected from the Bedgebury Pinetum and Forest Plots, including thirteen species of *Abies*, seven species of *Picea* and seventeen species of *Pinus*.

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EXPERIMENTAL

Chromatographic procedure

Thin-layer absorbent

Silica Gel G plates, 0.25 mm thick, were used. The activity of the plates was found to be important, and optimum results were obtained when the plates were heated for 1 h at 100° and stored in a desiccator before use. Alumina was found to give unsatisfactory separations.

Solvent system

Ethyl acetate–hexane (15:85).

Application of test materials

The preservatives were applied to the plates as 0.1 % solutions in chloroform. The volume of preservative solution applied was in excess of the lower limit of detectability of the locating reagents described below. With the commercial preservatives tested, these limits were: *o*-phenylphenol (*o*-PP), 5–10 μl; pentachlorophenol (PClP), tributyltin oxide (TBTO) and benzene hexachloride (BHCl) 50 μl; and polychloronaphthalene (PClN) 100 μl.

Development of the plates

A tank saturated with solvent vapour was used and the solvent front was allowed to advance 15 cm from the origin.

Methods of detection

0.1 % alcoholic solution of diiodofluorescein. The developed plates were sprayed with this alcoholic solution and exposed to bromine vapour. The plates were examined in daylight, when yellow spots were visible on a pink background, and in U.V. light.

0.1 % solution of dithizone in chloroform. This reagent is recommended for the detection of TBTO. The developed plates were sprayed with dithizone solution followed by an *N*/50 solution of EDTA.

Boron trifluoride–methanol complex. The plates were sprayed with the complex, heated at 100° and the fluorescence observed under U.V. light.

Potassium permanganate–sulphuric acid solution. The plates were sprayed with an aqueous solution of 0.2 % $KMnO_4$ in 0.04 % sulphuric acid.

Potassium ferricyanide reagent[4]. The plates were sprayed with a solution containing 0.05 g potassium ferricyanide and 1.0 g ferric chloride in 10 ml water.

Copper–pyridine reagent[6]. Concentrated sulphuric acid (110 ml) was diluted with 300 ml distilled water and added to a separately prepared solution containing 6 g copper sulphate ($CuSO_4 \cdot 5H_2O$) and 80 g pure pyridine in 114 ml distilled water.

Rhodamine 6G solution[5]. An aqueous solution containing 0.02 % Rhodamine 6G was sprayed on to the developed plates which were then examined under U.V. light and the visible spots were marked. The plates were then exposed to Br vapour and re-examined in U.V. light.

This reagent was also used in conjunction with diiodofluorescein spray. The developed plate was first sprayed with Rhodamine 6G solution and examined under

U.V. light. It was then sprayed with diiodofluorescein solution and re-examined under U.V. light before exposure to Br vapour followed by a final examination under U.V. light.

Preparation of wood extracts

Coarsely ground wood (10 g) was Soxhlet extracted for 4 h with 200 ml chloroform and the extract was concentrated to 10 ml on a rotary evaporator at 50° (1 g wood = 1 ml extract).

Gas–liquid chromatography

A Perkin Elmer Model F11 was used with a metal column 2 m long × 2.2 mm diameter, packed with 10% LAC-3-R-728 on Chromosorb W. A column temperature of 180° and a range of 2×10^2 were used.

Gas pressures were N 30 lb./sq.in., H 15 lb./sq.in. and air 25 lb./sq.in.

4 μl of a mixture of preservatives containing pentachlorophenol 0.3%, *o*-phenylphenol 0.01%, tributyltin oxide 0.1% and benzene hexachloride 0.1%, were injected with 20 μl wood extract. This was equivalent to 12 μg PClP, 0.4 μg *o*-PP and 4 μg of TBTO and BHCl in 0.2 g wood.

RESULTS AND DISCUSSION

Detection of preservatives on thin-layer chromatograms (Table I)

Sodium hydroxide–diazotized α-naphthylamine spray has been recommended for the detection of *o*-phenylphenol on TLC and dithizone solution for TBTO (the colours obtained being shown in Table I).

In our experiments the diiodofluorescein was more sensitive than dithizone for the detection of most of the commercial preservatives examined and also gave different colours with various preservatives under U.V. light.

The boron trifluoride–methanol complex was found to be much less sensitive than diiodofluorescein for the detection of the preservatives, but it could be used to locate four of the five compounds examined.

Universal indicator was used as a spray for the detection of all five compounds, but was non-specific and less sensitive than the other reagents used.

PClP, TBTO and *o*-PP could all be detected using $KMnO_4$–sulphuric acid, *o*-PP being the most readily located. This compound was also readily detected using potassium ferricyanide–ferric chloride reagent.

Copper–pyridine reagent, which was recommended for the detection of PClP, gave a non-distinctive brown colour, visible in daylight with this compound, whereas it gave a blue colour with PClN under U.V. light.

Diiodofluorescein was the most sensitive of the various reagents used for the detection of all five preservatives. It would detect 50–100 μg PClN and 50 μg PClP, TBTO and BHCl. It was particularly sensitive to *o*-PP, detecting spots containing 1–5 μg.

o-PP was the compound most readily identified in mixtures of preservatives, and was also easily detected in the presence of wood extracts. When mixed formulations of preservative containing *o*-PP are used on timber, determination of this one component could be used to assess the quantity of the preservative present.

TABLE I

REACTIONS OF FIVE WOOD PRESERVATIVE COMPOUNDS

Reagent	*Light**	*PClP*	*o-PP*	*TBTO*	*PClN*	*BHCl*
Dithizone	n	—	—	yellow	—	—
	U.V.	pink	blue	yellow-orange	blue	blue-green
Diiodofluorescein	n	—	yellow	—	—	—
	U.V.	absorp.	absorp.	yellow	orange + absorp.	yellow
Universal indicator		pH 4	pH 4	pH 6	pH 5	pH 5.5
$KMnO_4$–H_2SO_4	n	orange	orange	orange	—	—
	U.V.	absorp.	absorp.	absorp.	—	—
Boron trifluoride	U.V.	absorp.	absorp.	blue	blue	—
Ferric chloride–potassium ferricyanide	n	—	blue	—	—	—
Copper–pyridine	n	brown	—	—	—	—
	U.V.	—	—	—	blue	—
Diazotized sulphanilic acid		—	light brown	—	—	—
Rhodamine 6G	n	light purple	yellow-orange R_F 40	light purple R_F 5	light purple R_F 90	—
	U.V.	absorp.** R_F 0–40 R_F 50, R_F 55	absorp. R_F 40 yellow R_F 95	absorp. R_F 20, R_F 35 yellow R_F 5, R_F 85	absorp. R_F 75 orange R_F 90	light yellow R_F 67
Rhodamine 6G + bromine	n	purple	brown R_F 40	—	—	—
	U.V.	—	—	yellow at origin	light blue R_F 90	—

* n = examined in daylight; U.V. = examined in ultraviolet light.
** R_F = × 100.

Detection of *o*-PP with diiodofluorescein was found to be more convenient than using the leuco base dye, 4,4′-bis(dimethylamino)triphenylmethane, suggested for detection of 0.1 lb. of PClP per cu. ft. of wood[7].

Absorbents and solvent system (Table II)

Silica Gel G (STAHL) and alumina were used as absorbents, Silica Gel G giving a better resolution of the commercial preservative compounds examined. Silica Gel G also gave the most satisfactory separation of the constituents of the various wood extracts investigated.

Ethyl acetate–*n*-hexane (15:85) was selected as the most satisfactory solvent system, the mean R_F of the preservatives being shown in Table II[3]. R_F values were affected by the quantity of material applied to the plates, and by any interfering compounds applied with the preservative solution. Commercial preservatives are not pure chemicals, but contain varying amounts of impurities. Many of these impurities

TABLE II

MEAN R_F (× 100) OF PRESERVATIVES ON ALUMINA AND SILICA GEL G IN DIFFERENT SOLVENT SYSTEMS

Compound	*Chloroform–acetone*	*Ethyl acetate–n-hexane*				*Adsorbent layer*
	(9:1)	(1:9)	(1.5:8.5)	(2:8)	(3:7)	
PClP	0	0	0		0	alumina
		8	10	28	63	Silica Gel G
o-PP	70	61	50		74	alumina
		43	53	55	55	Silica Gel G
TBTO	70	43	28		75	alumina
		0	0	0/90	75	Silica Gel G
PClN	75	95				alumina
		80	90	88		Silica Gel G
BHCl	75	90	98		88	alumina
		58	75	73	67	Silica Gel G

were detected by TLC, particularly those in TBTO and PClP, but more impurities were detected using GLC. Pentachloronaphthalene gave only one spot, with a yellow red centre surrounded by a dark absorption ring when examined by TLC using the above system, followed by spraying with diiodofluorescein, whereas it gave up to eighteen different peaks when examined by GLC.

Detection of wood preservatives in wood extracts, using TLC (Table III, Fig. 1)

Chloroform was found to be a good solvent for the extraction of the preservatives from treated wood, extracting relatively small quantities of the naturally occurring phenols, compared with other organic solvents tested, *e.g.*, methanol.

Using TLC, it would be possible to detect at least four of the five preservatives in the majority of the nine woods examined (see Table III, Fig. 1). With most of the extracts, up to 100 μl (equivalent to 0.1 g wood) could be applied to the plates as a single spot, which would enable preservatives to be detected even when present in the wood at very low concentrations. The exceptions were Pitch Pine extract, which was too concentrated to give satisfactory separations when only 10 μl was applied, and Scots Pine extract, which gave unsatisfactory results when more than 25 μl were used.

Diidofluorescein was the most useful general detecting reagent for all five preservatives, but detection would not be possible unless there was a suitably high concentration of the preservatives. TBTO was found to be the most difficult compound to detect.

The best system for the detection of the preservatives was Rhodamine 6G/diiodofluorescein in which the plates were sprayed first with Rhodamine 6G, followed by

TABLE III

SUMMARY OF PRESERVATIVES DETECTABLE IN EXTRACTS OF COMMERCIAL WOODS USING TLC AND GLC

Detection: + = possible, — = impossible, +? = difficult.

Wood species	*Volume of extract applied (µl)*	*Preservatives*									
		PClP		*o-PP*		*TBTO*		*PClN*		*BHCl*	
		TLC	*GLC*	*TLC*	*GLC*	*TLC*	*GLC*	*TLC*	*GLC*	*TLC*	*GLC*
Araucaria angustifolia (Parana Pine)	100	+	+	+	+	+?	+	+	—	+	+
Juniperus communis (Western Red Cedar)	100	+?	—	+	+	+?	+	+	—	+?	+
Larix decidua (European Larch)	100	+?	—	+	+	+?	+	+	—	+	+
Pinus palustris (Pitch Pine)	10	?	—	?	—	?	—	+	—	+?	—
Pinus sylvestris (Scots Pine)	25	+	—	+	+	+	+*	+	—	+?	+
Pseudotsuga sp. (Douglas Fir)	100	+?	+	+	+	+	+?*	+	—	+	+
Tsuga heterophylla (Western Hemlock)	100	+?	+?	+	+?	+?	+	+	—	+?	+
Taxus sp. (Yew)	50–100	+?	+	+	+?	+	+	+	—	+	+
Quercus sp. (European Oak)	100	+?	+?	+?	+	—	+	+	—	+?	+

* Would be possible with lower quantities of wood extract present.

diiodofluorescein and exposure to bromine vapour, the chromatograms being examined in daylight and U.V. light after each stage of treatment.

Detection of *o*-phenylphenol was very sensitive, and identification of this compound in a sample of timber could be taken as proof that the wood had been treated with a mixed formulation of commercial preservatives.

The systematic study of the genera *Abies*, *Picea* and *Pinus* gave results which were comparable to those obtained with the nine commercial woods investigated (see Table IV).

Detection of preservatives in wood extracts, using GLC (Fig. 2)

A column packed with 10 % LAC-3-R-728 on Chromosorb W gave satisfactory separation of PClP, *o*-PP, TBTO and BHCl (see Fig. 2). Many commercial preservatives contain impurities and therefore give more than one peak on GLC, *e.g.* PClP and BHCl, but despite this detection was not difficult. The sample of PClN used,

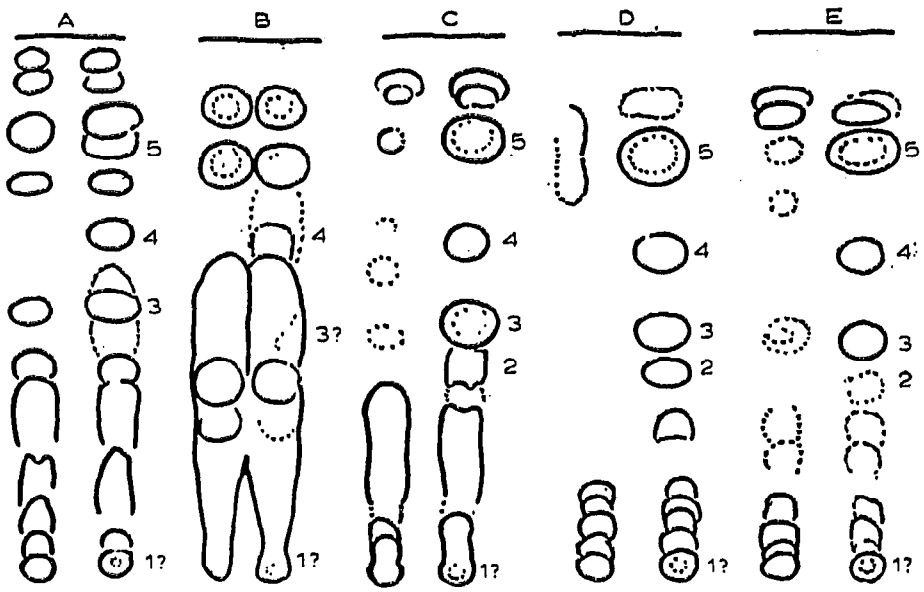

Fig. 1. TLC of the wood of (A) *Juniperus occident*; (B) *Pinus palustris*; (C) *Pinus sylvestris*; (D) *Pseudotsuga* sp.; (E) *Quercus* sp. 100 μl applied (except B = 10 μl). 1 = TBTO; 2 = PClP; 3 = *o*-PP; 4 = BHCl; 5 = PClN.

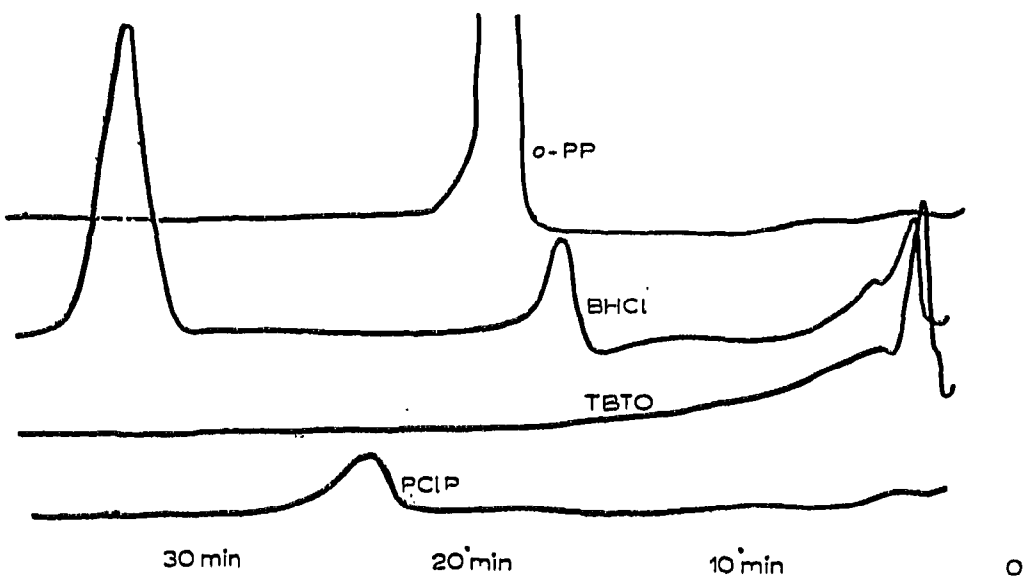

Fig. 2. GLC of *o*-phenylphenol, benzene hexachloride, tributyltin oxide and pentachlorophenol.

TABLE IV

FEASIBILITY OF DETECTION OF COMMERCIAL WOOD PRESERVATIVES IN SPECIES OF THE GENERA *Abies*, *Picea* AND *Pinus* BY TLC

+ = possible, — = not possible, +? = not always possible.

Genus and species	*PClP*	*o-PP*	*TBTO*	*PC'N*	*BHCl*
Abies					
bornmueliana	+	+	Difficult		+
cephalonica	+	+	Difficult	+	+
cilicia	+	+	Difficult	+	+
concolor	+	+	Difficult	+	+
delavayi	+	+	Difficult	+	+
engelmanii					
firma	+	+	Difficult	+	+
grandis	+	+	Difficult	+	+
homolepis	+	+	Difficult	+	+
lasiocarpa	+	+	Difficult	+	+
loviana	+	+	Difficilti	+	+
nordmaniana	+	+	Difficult	+	+
weitchii	+	+	Difficult	+	+
Picea					
abies	+	+	Difficult	—	+
aspera	+	+	Difficult	+	+
glauca	+?	+	Difficult	+	+
jezoensis-kondoensis	+	+	Difficult	—	+
mariana	+	+	Difficult	+	+
omorika	+?	+	Difficult	+	+
rubens	+	+	Difficult	+	+
Pinus					
banksiana	+	+	Difficult	+	+
contorta	+	+	Difficult	—	+
coulteri	+	+	Difficult	—	+
griffithii	+?	+	Difficult	—	+
mugo	—	—	Difficult	—	+
muricata	—	+	Difficult	+	+
nigra var. *caramanica*	+	+	Difficult	+?	+
nigra var. *cebenensis*	+?	+	Difficult	+	+
peuce	+	+	Difficult	+	+
pinaster	+	+	Difficult	—	+
ponderosa	+	+	Difficult	—	+
radiata	+	+	Difficult	—	+
resinosa	+	+	Difficult	—	+
strobus	+	+	Difficult	+?	+
sylvestris Darnaway	+	+	Difficult	+?	+
tabulaeformis	+	+	Difficult	—	+
virginiana	+	+	Difficult	+	+

however, gave eighteen peaks and therefore detection in mixtures was impossible.

BHCl and *o*-PP were readily identified, the lower limit of detectability with the latter being 1/10 to 1/20 of the lowest limit with any of the other compounds used.

The majority of woods did not contain materials which interfered with the GLC identification of the preservatives. Even with the highest concentration of wood extract used, there were few peaks which came within the retention time of the preservatives. One exception was Pitch Pine extract which contains a high concentration of resinous constituents which prevented the detection of preservatives, even when

1/10 to 1/20 of the quantity of wood extract was used. With the majority of extracts it was possible to detect single preservatives and mixtures of preservatives provided PClN was not included. If PClN was present alone, it was possible to detect it in many cases as there were few peaks derived from the wood extracts which corresponded to the eighteen peaks obtained with the PClN due to the interference of the PClN peaks with those from the other preservatives. An exception was the detection of *o*-PP when present in the wood in large quantities relative to the PClN content.

It was found that a combination of TLC and GLC provided a method for the detection of the presence or absence of the preservatives in the majority of woods examined.

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SUMMARY

The detection of five commercial wood preservatives in various wood extracts was investigated using thin-layer and gas–liquid chromatography. Studies were made on chloroform extracts of wood from 46 species, mainly from the coniferous genera *Abies*, *Picea* and *Pinus* using thin-layer chromatography. The preservatives tributyltin oxide, *o*-phenylphenol, benzene hexachloride, pentachlorophenol and polychloronaphthalene were best separated on Silica Gel G with ethyl acetate–*n*-hexane (15:85) as a running solvent. Diiodofluorescein was found to be a useful general locating reagent for all of the preservatives examined and *o*-phenylphenol was found to be more readily detected than the other preservative compounds, whereas tributyltin oxide was the most difficult to locate, and it was not possible to identify this compound in the presence of several of the wood extracts. Using gas–liquid chromatography, a column packed with 10% LAC-3-R-728 on Chromosorb W was found to be suitable for the identification of three or four of the preservatives, but the polychloronaphthalene sample gave eighteen peaks on the chromatogram, and this large number of impurities prevented identification of the polychloronaphthalene itself.

The suggested combination of thin-layer and gas–liquid chromatography provides a suitable method for the detection of the above preservatives in a large number of species of wood.

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