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Constituents of Pine Heartwood

XXV. * Investigation of Forty-Eight *Pinus* Species by Paper Partition Chromatography

GÖSTA LINDSTEDT and ALFONS MISIORNY

Organisk-kemiska Institutionen, Kungl. Tekniska Högskolan. Stockholm, Sweden

Previous parts of this series have dealt with the isolation of heartwood substances from a number of pines by conventional methods. In Part XX [1] the general methods for analysing pine heartwood extracts by paper chromatography were described, and it was there mentioned that a large number of pines had been investigated by this new technique.

The present paper describes the results of these investigations, which have been carried out on a total of forty-eight *Pinus* species including those which were studied by the earlier methods. It has been found that the use of paper chromatography generally reveals the presence of additional substances which could not be isolated by these methods; in one case only, that of strobochrysin [2] in *P. strobus*, was it not possible to identify on the chromatogram a phenol which had been isolated from the heartwood by other means.

The results are summarised in Table 1 (see below), in which the pine species are listed in the order given in Shaw's monograph [3]. A few pines which were regarded by Shaw to be subspecies or varieties of other pines have been listed as separate species according to more recent literature [4,5]. The pines that have not been investigated are omitted from the table.

SPOTS BELONGING TO UNKNOWN SUBSTANCES

As described in previous papers, some of the new substances discovered by the use of paper chromatography have later been actually isolated from the heartwood extracts. There are, however, several such substances which are visible on the chromatogram but have not yet been isolated. These are:

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Table 1. Heartwood constituents of the genus P i n u s.

+ = isolated from the heartwood
− = not found by paper chromatography
no sign = not investigated
+ = identified by paper chromatography
? = identification uncertain

PSM = Pinosylvin monomethyl ether
DHPS = Dihydropinosylvin
DHPSM = Dihydropinosylvin monomethyl ether

		Species	Pinosylvin	PSM	DHPS	DHPSM	Pinocembrin	Chrysin	Pinostrobin	Tectochrysin	Pinobanksin	Strobobanksin	Strobopinin	Cryptostrobin	Unknown compounds (see text) D	E	F	G
A. Haploxylon																		
Subsection *Cembra*	*Cembrae*	*P. koraiensis* Sieb. & Zucc.	+	+	+	+	+	+	+	+	+	−	−	−	+	−	−	−
		P. cembra L.	+	+	+	+	+	+	+	+	?	−	−	−	+	−	−	−
		P. albicaulis Engelm.	+	+	+	+	+	+	+	+	+	−	−	−	+	−	−	−
	Flexiles	*P. flexilis* James	?	+	+	+	+	+	+	+	+	−	−	−	+	−	−	−
	Strobi	*P. ayacahuite* Ehrenb.	+	+	+	+	?	+	?	+	+	−	−	−	−	−	−	−
		P. Lambertiana Dougl.	−	−	−	−	+	?	+	+	+	+	+	?	+	−	−	−
		P. parviflora Sieb. & Zucc.	+	+	+	+	+	+	−	−	+	−	+	+	+	−	−	−
		P. peuce Griseb.	−	−	−	−	+	+	+	+	?	−	+	+	+	−	−	−
		P. Griffithii M'Clelland (= *P. excelsa* Wall.)	+	+	−	+	+	+	+	+	+	−	−	−	−	−	−	−
		P. monticola Dougl.	+	+	−	+	+	+	+	+	?	−	+	+	+	−	−	−
		P. strobus L.	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−
	Cembroides	*P. cembroides* Zucc.	−	+	−	+	+	+	+	?	?	−	−	−	−	+	+	−
Subsection *Paracembra*	*Gerardianae*	Not investigated																
	Balfourianae	*P. Balfouriana* A. Murr.	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	−
		P. aristata Engelm.	?	+	−	+	+	+	+	+	?	−	−	−	+	+	−	−
B. Diploxylon																		
Subsection *Parapinaster*	*Leiophyllae*	*P. leiophylla* Schlecht. & Cham.	+	+	−	−	+	−	−	−	+	−	−	−	+	−	−	−
		P. Lumholtzii Robins. & Fern.	+	+	?	+	+	−	−	−	+	−	−	−	−	−	−	−
	Longifoliae	*P. canariensis* Smith	+	+	−	−	+	−	−	−	+	−	−	−	−	+	−	−
	Pineae	*P. pinea* L.	+	+	−	−	+	−	−	−	+	−	−	−	−	+	−	−

		Species	Pinosylvin	PSM	DHPS	DHPSM	Pinocembrin	Chrysin	Pinostrobin	Tectochrysin	Pinobanksin	Strobobanksin	Strobopinin	Cryptostrobin	Unknown compounds (see text) D	E	F	G
Subsection *Pinaster*	*Laricines*	*P. resinosa* Ait.	+	+	–	–	?	–	–	–	?	–	–	–	–	+	+	–
		P. massoniana Lamb.	+	+	–	–	+	–	–	–	+	–	–	–	+	+	+	+
		P. densiflora Sieb. & Zucc.	+	+	–	–	+	–	–	–	+	–	–	–	+	+	+	–
		P. sylvestris L.	+	+	–	–	+	–	–	–	+	–	–	–	–	+	–	–
		P. mugo Turra (= *P. montana* Mill.)	+	+	–	–	+	–	–	–	?	–	–	–	+	+	+	?
		P. nigra var. *Poiretiana* (= *calabrica*) Schneid.	+	+	–	–	+	–	–	–	–	–	–	–	–	+	+	?
		P. nigra var. *austriaca* (Hoess) Badoux	+	+	–	–	+	–	–	–	–	–	–	–	+	+	+	–
	Australes	*P. Montezumae* Lamb.	+	+	–	–	+	–	–	–	+	–	–	–	–	+	+	–
		P. ponderosa Dougl.	+	+	–	–	+	–	–	–	+	–	–	–	+	+	+	–
		P. Jeffreyi Balf.	+	+	–	–	+	–	–	–	+	–	–	–	+	+	+	+
		P. occidentalis Swartz	+	+	–	–	+	–	–	–	+	–	–	–	–	+	+	+
		P. palustris Mill.	?	+	–	–	+	–	–	–	+	–	–	–	+	+	+	+
		P. caribaea Morelet	–	+	–	–	+	–	–	–	+	–	–	–	+	+	+	+
		P. taeda L.	+	+	–	–	+	–	–	–	+	–	–	–	+	–	–	–
		P. glabra Walt.	+	+	–	–	+	–	–	–	+	–	–	–	+	+	+	+
		P. echinata Mill.	+	+	–	–	+	–	–	–	+	–	–	–	+	+	?	+
	Insignes	*P. halepensis* Mill.	+	+	–	–	+	–	–	–	+	–	–	–	+	+	+	+
		P. pinaster Ait.	+	+	–	–	+	–	–	–	+	–	–	–				
		P. virginiana Mill.	?	+	–	–	+	–	–	–	+	–	–	–	?	+	+	–
		P. clausa Vasey*	+	+	–	–	+	–	–	–	+	–	–	–	–	–	–	–
		P. rigida Mill.	+	+	–	–	+	–	–	–	+	–	–	–				
		P. Pungens Lamb.	+	+	–	–	+	–	–	–	+	–	–	–	+	+	+	+
		P. Banksiana Lamb.	+	+	–	–	+	–	–	–	+	–	–	–	+	+	?	–
		P. contorta var. *latifolia* Engelm.	+	+	–	–	+	–	–	–	+	–	–	–	+	+	–	+
		P. muricata D. Don	+	+	–	–	+	–	–	–	+	–	–	–				
		P. attenuata Lemm.	?	+	–	–	+	–	–	–	+	–	–	–	+	+	–	?
		P. radiata D. Don	+	+	–	–	+	–	–	–	+	–	–	–	+	+	+	–
		P. radiata var. *insignis* Dougl.	+	+	–	–	+	–	–	–	+	–	–	–				
	Macrocarpae	*P. sabiniana* Dougl.	+	+	–	–	+	–	–	–	+	–	–	–	+	+	+	+
		P. Coulteri D. Don	+	+	–	–	+	–	–	–	+	–	–	–	+	+	+	+

* One sample of *P. clausa* also contained pinostrobin and 3,5-dihydroxy-7-methoxyflavanone, see Part XXII[6].

1. *Dihydropinosylvin?* A spot with $R_F \sim 0.1$ giving a red colour at the first moment of spraying with benzidine reagent has been observed on the chromatograms of some pines from the subgenus *Haploxylon*. Since an authentic specimen of dihydropinosylvin gives a spot with identical properties [1], it seems very probable that the spot should be due to this substance, especially as its monomethyl ether has been found in several pines. (See Table 1 and Part XXIII [7]). To judge from the colour intensity of the spot, the content of dihydropinosylvin is always very low, and no efforts have been made to isolate the substance.

2. *Compound A.* A spot with $R_F \sim 0.1$ which is stained yellow by benzidine reagent. This compound has been found in *P. Massoniana*, *P. densiflora*, *P. Montezumae* and *P. occidentalis*, all from the subgenus *Diploxylon*.

3. *Compound B.* A pale red spot appearing just below the pinobanksin spot ($R_F \sim 0.2$). It is not due to chrysin and has been observed in a few *Diploxylon* pines.

4. *Compound C.* A spot with $R_F \sim 0.4$ giving a red colour with benzidine reagent. This spot has only been observed on chromatograms from *P. koraiensis* and *P. Griffithii*.

5. *Compound D.* A rather sharp spot with $R_F \sim 0.45$ in standard solvent, giving a bluish-green fluorescence when the unsprayed paper is exposed to ultraviolet light but giving no distinct colour with benzidine reagent. This spot has been observed in most pines from both subgenera (see Table 1). It is always strongest in the sodium carbonate fraction.

6. *Compound E.* A large and trailing spot with $R_F \sim 0.55$ in standard solvent. It is not visible after spraying with benzidine reagent but gives a faint bluish fluorescence in ultraviolet light which changes to very intense blue after spraying with sodium carbonate. This compound has been found in most pines from the subgenus *Diploxylon* but only in three *Haploxylon* pines, all of which belonging to the subsection *Paracembra*. (See Table 1.)

7. *Compound F.* A small crescent-shaped spot with the same fluorescence as E, which appears just below the spot of pinosylvin monomethyl ether ($R_F \sim 0.73$). It is found in roughly the same pines as E. (See Table 1.) Both these compounds accumulate in the strong alkali fraction.

8. *Compound G.* A spot having $R_F \sim 0.85$ in ligroin-ether (5 : 1), giving a yellowish fluorescence in ultraviolet light after spraying with sodium carbonate. It accumulates in the strong alkali fraction and has only been found in the subgenus *Diploxylon* (see Table 1).

The chromatograms of *P. palustris* contain one spot which seems to be unique for this species. It is stained pale red by benzidine reagent and has $R_F \sim 0.9$ but does not fluoresce under the quartz lamp. It accumulates in

the sodium carbonate and 0.2 % sodium hydroxide fractions. This compound may possibly be identical with "pinopalustrin", a phenolic lactone of the lignan type which has recently been isolated from stumps of *P. palustris* by Harris. (Private communication from Dr. C. G. Harris to Prof. H. Erdtman.)

Finally, in the strong alkali fraction from *P. Coulteri*, there is a compound which gives a reddish-brown spot with benzidine reagent, having $R_F \sim 0.8$. In contrast to dihydropinosylvin monomethyl ether, this spot requires some time to become visible. Traces of a similar spot can be observed on the chromatogram of *P. sabiniana*.

WATER-SOLUBLE COMPOUNDS

The isolation of *l*-arabinose from the heartwood extract of *P. sylvestris* was described by Erdtman in Part IV of this series [8]. Subsequently, the same sugar has been isolated from several other pines. In the present investigation, the water-soluble fraction of the acetone extract from each pine was investigated for sugars by paper chromatography. The presence of arabinose could be demonstrated for all pines investigated except *P. koraiensis* and *P. parviflora*. In addition to the arabinose spot, the chromatograms often showed a second spot, belonging to glucose.

Pinitol, which has been isolated from some pines belonging to the subgenus *Haploxylon*, is more difficult to identify on the paper chromatogram. Potassium permanganate in sodium carbonate solution [9] and ammoniacal silver nitrate have been tried as spraying reagents, but neither of them gives a sufficiently sure identification of pinitol, especially as its R_F value does not differ very much from that of glucose.

PINOSYLVIN DIMETHYL ETHER

A small quantity of pinosylvin dimethyl ether was once isolated from *P. nigra* by Erdtman [10]. In ligroin, this compound has $R_F \sim 0.9$ and can be identified with the aid of its fluorescence in ultraviolet light. The neutral fractions of the pines available here have been investigated in that way. In most cases, a spot similar to that of pinosylvin dimethyl ether was found on the chromatograms, both from *Haploxylon* and *Diploxylon*. The identification is, however, very uncertain, the fluorescence of pinosylvin dimethyl ether being much weaker than that of other spots in the neighbourhood. (The neutral fractions often fluoresce even in daylight.) Therefore, no list of the pines giving a positive test will be published here.

INVESTIGATION OF SAPWOOD

When stained with diazotised benzidine reagent, the sapwood is only slightly yellow coloured, thus indicating that its content of phenols is very low. The heartwood always acquires a more or less strongly red colour by the same treatment [11]. Traces of the heartwood phenols can, however, be found by paper chromatography even in sapwood extracts. Table 2 shows the result of an investigation of the sapwood of nine pine species.

Table 2. Investigation of sapwood by paper chromatography.

Species	Pinosylvin	PSM	DHPSM	Pinocembrin	Chrysin	Pinostrobin	Pinobanksin	Arabinose	Glucose	Unknown compounds	
										D	E
A. Haploxylon											
P. albicaulis	−	+	+	−	−	−	−	+	+	+	−
P. Griffithii	−	+	+	−	−	−	+	+	+	−	+
P. monticola	−	+	−	+	−	+	−	+	+	+	−
P. strobus	−	+	+	+	+	+	+	?	+	−	−
P. aristata	−	+	+	−	−	−	−	+	+	+	+
B. Diploxylon											
P. sylvestris	+	+	−	−	−	−	−	+	+	+	−
P. mugo	+	+	−	+	−	−	−	+	?	+	+
P. Banksiana	+	+	−	+	−	−	+	+	+	+	+
P. contorta	−	−	−	+	−	−	−	+	?	+	−

The content of phenols in the sapwood is always very low, but the content of sugars and compounds D and E seems to be comparable with that in the heartwood, to judge from the colour intensity of the spots. These substances, therefore, cannot be regarded as true heartwood constituents.

INVESTIGATION OF BARK

The bark of three species, *P. strobus*, *P. sylvestris* and *P. Banksiana*, has also been investigated. In no case could any traces of the heartwood phenols be discovered on the chromatograms. The initial spot usually gives a strong red or yellowish-brown colour with diazotised benzidine, indicating the presence of phenolic products with $R_F = 0$ in standard solvent.

EXPERIMENTAL

The amount of heartwood from each pine used for the investigation was 20—30 g, if such a quantity was available. In some cases, however, only 0.5—1 g were used. The finely-ground heartwood was extracted with ether in a Soxhlet apparatus for four hours, then dried in the air and extracted with acetone for another four hours. Both extracts were tested on the paper chromatogram. If the ether extract contained any appreciable amounts of heartwood phenols, it was concentrated to a small volume and precipitated with light petroleum. The insoluble part was then separated, dissolved in ether and combined with the ether solution of the acetone extract.

The acetone extract was concentrated to a small volume and the residue treated with a few ml of water. The water solution (W) was used for the sugar test (see below). The water-insoluble part was dissolved in ether, filtered and divided into fractions by shaking with sodium bicarbonate, sodium carbonate, 0.2 % sodium hydroxide and 4 % sodium hydroxide. The bicarbonate extraction was sometimes omitted. Each extract was acidified and taken up in ether. These ether solutions were used for the paper chromatograms; their concentrations often had to be adjusted by dilution or evaporation of the solvent in order to get good chromatograms. Two chromatograms with standard solvent [1] were run for each fraction. One of these was stained with benzidine reagent [1], and the other observed under the quartz lamp before and after spraying with 5 % sodium carbonate [12]. The two-dimensional technique and the chromatograms for identification of tectochrysin and pinostrobin described in Part XX [1] were also used. The neutral fractions were chromatographed in ligroin for the detection of pinosylvin dimethyl ether (see above).

The W fraction was concentrated in a vacuum to a small volume, and a paper chromatogram of this solution was run for 16 hours, using ethyl acetate-acetic acid-water (3 : 1 : 3) as the solvent [13]. Eight chromatograms were run simultaneously on a 25 × 40 cm sheet (Munktell OB). One of these contained a reference mixture of arabinose and glucose. The solvent was allowed to drip off the lower edge of the paper, which had been cut like the teeth of a saw. The spraying reagent used was aniline hydrogen phtalate [14], which gives reddish-brown spots for pentoses and greyish spots for aldohexoses after drying at 105°.

SUMMARY

The heartwood constituents of forty-eight *Pinus* species have been investigated by the aid of paper chromatography.

The results will be discussed in a succeeding paper.

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