

Constituents of Pine Heartwood

IX.* The Heartwood of *Pinus montana* Mill

GÖSTA LINDSTEDT

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

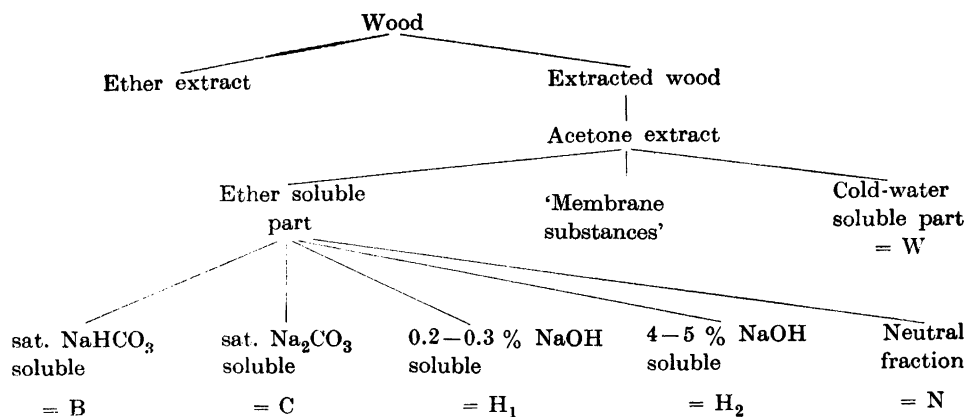
Some years ago, an investigation of the heartwood constituents of a few *Pinus* species was carried out by Erdtman¹. All these pines were found to contain pinosylvin (3,5-dihydroxistilbene) or its monomethyl ether. Most of them also contained flavone or flavanone derivatives, and in *P. cembra* and *P. strobus*, both belonging to the section *Haploxyton*, the heartwood yielded pinitol in addition to phenolic derivatives. The presence of phenols in the heartwood can be demonstrated by staining a freshly-cut section through the stem with diazotised benzidine solution. In a few seconds the heartwood turns more or less dark red (formation of azo dyes), whereas the sapwood, containing little or no phenolic substances, shows only a slight yellowish colour.

Although the phenols are ether-soluble, they cannot be extracted from the wood with ether. They are, however, easily extracted with acetone or alcohol. This is due to the presence of ether-insoluble 'membranes' which are supposed to surround the phenols in the wood, and thus prevent them from being extracted with ether². The 'membrane substances' can be precipitated from the acetone extract by ether. If the acetone extraction of the wood is preceded by an ether extraction, the greatest part of the resinous substances is removed by the ether, and isolation of crystalline products from the acetone extract is facilitated. *P. strobus*, however, behaves differently^{1a}. Most of its phenolic heartwood constituents were found in the ether extract, and the quantity of 'membrane substances' was relatively small.

An investigation of pine heartwoods is now being continued by the writer, and this is the first in a series of papers describing the heartwood constituents of several *Pinus* species from both the *Haploxyton* and the *Diploxyton* sections.

The extractions were carried out according to the general scheme given below:

* VIII. Erdtman, H. *Svensk Kem. Tid.* 56 (1944) 134.



The heartwood of *P. montana* (European pine, section *Diploxydon*) has already been briefly investigated by Erdtman^{1c}, who isolated pinocembrin (5,7-dihydroxyflavanone) and pinosylvin monomethyl ether from the acetone extract. In addition to these substances, pinosylvin was found in the present investigation.

The ether extract always contains small amounts of phenolic substances which have been extracted because some of the «membranes» were destroyed during the grinding². The phenols in question are almost insoluble in light petroleum, but most of them can be dissolved in boiling water. A rough estimation of the amount of phenols in the ether extract can be made by precipitating with light petroleum and extraction of the precipitate with boiling water. Thus the ether extract from *P. montana* yielded 0.5% of a phenolic fraction. This was not investigated any further. The study of the resin acids and other constituents of the ether extract lies beyond the scope of this investigation.

The sodium carbonate fraction of the acetone extract yielded pinocembrin, m. p. 194–195°*, $[\alpha]_D^{20} - 54.5^\circ$ (in methanol). (Erdtman reported -45.5° and a few lower values^{1b}.) The pinocembrin isolated during the present investigation was purified only by recrystallisation or adsorption, whereas earlier preparations of pinocembrin were purified also by vacuum sublimation. Control experiments have shown that vacuum-sublimated pinocembrin may have any specific rotation between zero and -50° and a m. p. between 195 and 200°. It is evident that the low rotation previously reported for pinocembrin is due to partial racemisation during the sublimation. The pinocem-

* All melting points uncorrected.

brin isolated from about ten different pine species had specific rotations between -54 and -57° . (See forthcoming papers.) It is probable, therefore, that this pinocembrin represents pure laevorotary 5,7-dihydroxiflavanone.

From the 0.3 % sodium hydroxide fraction of the acetone extract, pinosylvin was isolated, and the 5 % sodium hydroxide extract yielded pinosylvin monomethyl ether.

3.0 kg of heartwood yielded 270 g of ether extract (9 %), 2.4 g of pinocembrin (2.0 g of which were obtained in a pure state) (0.08 %), 1.4 g of pinosylvin (0.05 %) and 3.1 g of pinosylvin monomethyl ether (0.1 %).

The content of pinosylvin phenols in the heartwood of this specimen of *P. montana* seems to be low. *P. sylvestris* often contains one per cent or more of pinosylvin and its monomethyl ether. Too much stress, however, should not be laid on the quantitative yields in this investigation, since only one tree of each species has been used. An investigation of several hundred specimens of *P. sylvestris* seems to indicate that the phenol content shows great individual variations (Erdtman and Frank, to be published).

EXPERIMENTAL

The pine used for the investigation was from Omberg, Sweden. The extraction was started about three weeks after the tree had been cut down. The heartwood was stained dark red when treated with diazotised benzidine solution.

The heartwood was cut to pieces of 3–4 cm length and about 5 mm diameter, allowed to dry in the air for some days, and then ground to pieces of 2–3 mm size in a Wiley mill (weight of air-dried wood 3 kg) and extracted with ether in a stainless-steel percolator for 24 hours. The ether extract was concentrated to a yellowish-brown syrup (270 g), which crystallised entirely in a few days. 3.44 g of the ether extract were treated with 50 ml of light petroleum, which left 0.53 g undissolved. This residue was a colourless solid, melting gradually at $90-100^\circ$. 3.0 % of this substance could be extracted with boiling water. The aqueous extract deposited a yellow resinous precipitate when cooled. The alcoholic solution of this precipitate gave a very faint yellowish-brown colour with ferric chloride.

After this extraction, the ether adsorbed on the wood meal was evaporated by indirect heating with steam, and the wood extracted with acetone for about 60 hours. The acetone extract was concentrated to a dark brown syrup, and treated with 400 ml of ether. A sticky precipitate of ether-insoluble 'membrane substances' was filtered off. The filtrate was shaken four times with about 200 ml of saturated sodium bicarbonate solution and then with saturated sodium carbonate solution (4×200 ml). Bicarbonate extract = B, carbonate extract = C.

The ether solution was then diluted to 600 ml and shaken twice with 500 ml of 0.3 % sodium hydroxide solution (= H_1) and, finally, twice with 200 ml of 5 % sodium hydroxide (= H_2). Remaining ether solution = N.

B yielded a very small amount of sticky precipitate when acidified. This fraction was discarded.

C was acidified with dilute sulphuric acid (brown, sticky precipitate) and extracted with ether. The yellow ether solution was dried over sodium sulphate and filtered through aluminium oxide. A dark brown zone was formed in the upper part of the column, but the filtrate was still yellow. It was evaporated to dryness. A brown crystalline cake was formed, which was recrystallised from toluene, yielding pale yellow crystals melting at 183–188° and a brown oil, from which crystals melting at 191–192° could be obtained by extraction with boiling water (3 l). These crystals were combined and recrystallised twice from 50 % acetic acid, yielding colourless needles, m. p. 194–195°, no melting-point depression with pinocembrin from *P. Banksiana*. Yield 2.0 g. $[\alpha]_D^{20} - 54.5^\circ \pm 0.5^\circ$. (Methanol, $c = 4.0$.)

From the mother liquors 0.4 g of less pure pinocembrin was obtained.

*H*₁ was acidified and shaken with ether. The ether solution was dried over sodium sulphate and the ether evaporated. The remaining reddish-brown oil was left standing overnight with a small amount of benzene. The next day light brown crystals had formed. They were separated, dissolved in ether and filtered through aluminium oxide, which removed most of the colour of the solution. The filtrate was concentrated to an almost colourless crystalline cake, from which pure pinosylvin was obtained after vacuum sublimation and two recrystallisations from xylene. Yield 1.4 g, m. p. 155–156°. A mixture with pinosylvin from *P. sylvestris* melted at the same temperature.

*H*₂ was acidified with dilute sulphuric acid, extracted with ether, and the ether solution filtered through aluminium oxide after being concentrated to 100 ml. This treatment removed most of the colour of the solution. On evaporation, a yellow crystalline cake of crude pinosylvin monomethyl ether was obtained, which was purified by two recrystallisations from 50 % acetic acid. Yield 3.1 g, m. p. 120–121°.

N was concentrated to a brown turpentine-smelling oil (2 g). No pinosylvin dimethyl ether could be found in this fraction by treatment with 1,3,5-trinitrobenzene in methanol solution^{1c}.

SUMMARY

Pinocembrin (5,7-dihydroxiflavanone), pinosylvin (3,5-dihydroxistilbene), and pinosylvin monomethyl ether have been isolated from the heartwood of *Pinus montana* Mill.

The author is indebted to Professor H. Erdtman for the proposal of the subject of this investigation and for collecting the wood.

The investigation was facilitated by a grant from *Statens Tekniska Forskningsråd*.

REFERENCES

1. Erdtman, H. *Svensk Kem. Tid.* 56 (1944) 2(a), 26(b), 95(c).
2. Häggglund, E., Holmberg, J., and Johnson, T. *Svensk Papperstidn.* 39 (1936) Specialnummer 37.

Received June 22, 1949.