

Constituents of Pine Heartwood

XVI.* The Heartwood of *Pinus virginiana* Mill.

GÖSTA LINDSTEDT

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

Pinus virginiana, 'Scrub Pine', growing in the southeastern part of the United States of America, is a pine belonging to the group *Insignes* of the section *Diploxylon*¹. This group contains, among other species, *P. Banksiana*, *P. contorta* and *P. radiata*, the heartwood constituents of which have been previously investigated²⁻⁴.

The heartwood was extracted with ether and acetone in the usual way (see Part IX⁵). The ether extract (11 % of the heartwood) crystallised partly. Treatment with light petroleum separated it into a soluble and an insoluble fraction. The insoluble fraction was found to contain pinobanksin. No other crystalline products could be isolated from it. The water-soluble fraction of the acetone extract contained comparatively large quantities of *l*-arabinose, and the sodium carbonate fraction yielded pinobanksin (3—4 times the quantity found in the ether extract). Pinocembrin was found in the 0.2 % sodium hydroxide fraction, and also, in a small quantity, in the 4 % sodium hydroxide fraction. This fraction consisted mainly of resinous products, from which 60 mg of pinosylvin monomethyl ether could be isolated after a very tedious process of separation.

The total yields from 6.3 kg of air-dried heartwood were:

'Membrane substances'	7.5 g (0.12 %)
<i>l</i> -Arabinose	8.3 g (0.13 »)
Pinobanksin	15.2 g (0.24 »)**
Pinocembrin	5.6 g (0.09 »)
Pinosylvin monomethyl ether	0.06 g (0.001 »)
Neutral fraction of acetone extract	14.9 g (0.24 »)
» » » ether »	3.8 g (0.06 »)

* XV. *Acta Chem. Scand.* 3 (1949) 1375.

** 11.4 g of pinobanksin were obtained from the acetone extract. Only a small part (about 13 %) of the ether extract was investigated. The calculated yield of pinobanksin from the entire ether extract was 3.8 g.

Just as its relatives *P. Banksiana*, *P. radiata* and *P. contorta*, *P. virginiana* also contains pinobanksin (probably 3,5,7-trihydroxyflavanone). The yield from the specimen used for the present extraction was better than from any pine previously investigated. The very low content of pinosylvin monomethyl ether is remarkable. Due to the lack of additional wood samples, it is impossible to say if this anomaly is characteristic of the whole species, or if the specimen investigated here just happened to be extraordinarily poor in pinosylvin monomethyl ether.

EXPERIMENTAL

The wood used for the investigation came from a tree grown at Lee Experimental Forest, Buckingham, Virginia, U.S.A. The heartwood gave a rather weak red colour when stained with diazotised benzidine solution.

6.3 kg of air-dried heartwood were extracted with ether for 24 hours and then with acetone for 48 hours. The ether extract (686 g) was a brown syrup which partly crystallised. 283 g of it were treated with light petroleum (1.5 l), leaving an insoluble yellowish precipitate which was collected, washed, and dried. Yield 22.2 g. It melted gradually at 70–100° under decomposition. Its solution in acetone gave no precipitate with cyclohexylamine, but the light petroleum solution gave a thick precipitate, indicating that it contained resin acids.

Part of the insoluble fraction (7 g) was extracted with boiling water, and the extract cooled and shaken with ether. The ether was evaporated, yielding a semi-crystalline residue, from which pure pinobanksin (0.4 g) was obtained after one recrystallisation from 50 % acetic acid and two from toluene. The m. p. of the substance was 176–177°.* A somewhat better yield of pinobanksin (0.5 g) was obtained from another 7 g portion of the insoluble fraction, which was dissolved in ether and divided into fractions by shaking with sodium bicarbonate, sodium carbonate, 0.2 % sodium hydroxide and 4 % sodium hydroxide solutions in the usual way. The sodium carbonate fraction was acidified and the resulting brown precipitate extracted with ether. The ether solution was concentrated and the residue recrystallised from 50 % acetic acid and from toluene to yield pure pinobanksin. The 0.2 % sodium hydroxide fraction yielded a brown oil which did not crystallise, and the 4 % sodium hydroxide fraction a very small quantity of a brown sticky product. The remaining 'neutral fraction' was concentrated to a brown turpentine-smelling syrup (0.5 g).

The acetone extract was concentrated to a brown resinous product and a small volume of water solution. The water phase (= W) was separated by decantation, and the resin treated with 400 ml of ether to precipitate 'membrane substances'. They were separated, dried in the air and stirred with cold water (100 ml). The suspension was then filtered, and the filtrate combined with W. The 'membrane substances' were air-dried, yielding 7.5 g of a light brown powder.

The ether solution was first shaken with water (100 ml), which was combined with W. It was then divided into fractions by successive shaking with saturated sodium bicarbonate (3 × 250 ml), saturated sodium carbonate (3 × 500 ml), 0.2 % sodium

* All melting points uncorrected.

hydroxide (2×200 ml), and 4 % sodium hydroxide (2×200 ml) solutions. The fractions are referred to as B, C, H_1 , and H_2 , respectively. The remaining ether solution, containing neutral products, was concentrated to a yellowish-brown oil of low viscosity (14.9 g), which had a turpentine-like odour and showed a strong fluorescence in ultra-violet light.

W was concentrated by vacuum distillation, yielding a brown syrup, which was dissolved in boiling ethanol. The extract was concentrated and cooled, yielding a colourless crystalline precipitate, which was separated and recrystallised twice from ethanol. Yield, 8.3 g of *l*-arabinose, m. p. $159-161^\circ$. $[\alpha]_D^{20} + 106^\circ \pm 1^\circ$ (equilibrium rotation in water, $c = 3.0$). The sugar gave a crystalline precipitate with *p*-bromophenylhydrazine in acetic acid solution. No additional crystalline products could be obtained from the filtrate after this precipitation.

B was acidified and extracted with ether. The ether solution was concentrated to a yellowish sticky product (12 g) which did not crystallise.

C: The sodium carbonate solution deposited a precipitate, consisting of yellow crystals and a brown resinous, semi-solid product. The entire precipitate (C_1) was separated from the solution, which was then extracted with ether. Ether extract = C_2 . The carbonate solution was then acidified and extracted with ether again. This ether solution yielded a yellow solid after evaporation of the ether. A small quantity of colourless crystals (0.7 g) could be separated from it. They melted at $133-140^\circ$ and gave a yellow precipitate with saturated sodium carbonate, indicating the presence of pinobanksin. This fraction was not investigated further.

C_1 was acidified and extracted with ether. The brown insoluble residue was dried in the air and extracted with ether again in a Soxhlet apparatus for about 90 hours, leaving 7.0 g of a brown insoluble powder. Further extraction with ether was of no effect. The combined ether extracts were dried over anhydrous sodium sulphate and the ether evaporated. The residue was a yellow crystalline product which was recrystallised from toluene, dissolved in ether, and decolourised by filtration through aluminium oxide. The filtrate was evaporated to dryness and the crystals recrystallised from toluene once again, yielding pale yellow crystals melting at $174-175^\circ$. After one recrystallisation from methanol-water, the m. p. was raised to $175-177^\circ$ and was not depressed on admixture of pinobanksin from *P. Banksiana*. $[\alpha]_D^{20} + 13.5^\circ \pm 0.5^\circ$ (methanol, $c = 3.6$). Yield, 11.4 g. The toluene mother liquors were concentrated by vacuum distillation and the residue recrystallised from 50 % acetic acid, yielding crude pinocembrin (2.5 g), m. p. $191-193^\circ$, which was combined with the corresponding fraction from H_1 .

C_2 was dried over anhydrous sodium sulphate and concentrated, yielding a yellow crystalline residue, which was recrystallised from 50 % acetic acid. A small quantity of crude pinocembrin was obtained. It was combined with H_1 .

H_1 : The 0.2 % sodium hydroxide extract was acidified and extracted with ether. The ether solution was dried over anhydrous sodium sulphate and the solvent evaporated. The pale yellow crystalline residue was recrystallised from 50 % acetic acid, yielding pale yellow crystals, m. p. $190-192^\circ$. This substance was combined with the crude pinocembrin from *C* and H_2 , dissolved in ether, and decolourised by filtration through aluminium oxide. The filtrate was evaporated to dryness and the residue recrystallised twice from 50 % acetic acid. Yield, 5.6 g of pinocembrin, m. p. $194-195^\circ$. $[\alpha]_D^{20} - 54^\circ \pm 1^\circ$ (methanol, $c = 2.1$).

H_2 : The alkaline solution was acidified and extracted with ether. The ether extract was dried and concentrated to a yellowish-brown syrup, which showed some tendency to crystallise. After treatment with a little ether, an insoluble precipitate could be separated. It melted at 187–189° and was combined with the crude pinocembrin from H_1 . The ether filtrate was concentrated to a syrup again, which did not crystallise in a week. It was then distilled in a vacuum, yielding a reddish-brown syrupy distillate. To remove the last traces of pinocembrin, this distillate was dissolved in ether and shaken with 0.1 % sodium hydroxide solution. The ether solution was then dried and filtered through aluminium oxide, which adsorbed most of its colour. After concentration, the ether filtrate deposited a small amount of a crystalline substance, m. p. 112–119°. After recrystallisation from chloroform-light petroleum, colourless crystals (60 mg), m. p. 118–120°, were obtained. A mixture with pinosylvin monomethyl ether melted at the same temperature. The remaining yellow syrup did not deposit any more crystals.

SUMMARY

The heartwood of *Pinus virginiana* Mill. has been investigated. *l*-Arabinose, pinobanksin (probably 3,5,7-trihydroxyflavanone), pinocembrin (5,7-dihydroxyflavanone) and a very small quantity of pinosylvin monomethyl ether were isolated from it.

The author is indebted to Mrs. B. Strömgren for skilful assistance with the experimental work and to Dr. I. T. Haigh, Southeastern Forest Experimental Station, Asheville, North Carolina, U.S.A., for supplying the wood. The investigation was facilitated by a grant from *Fonden för Skoglig Forskning*.

REFERENCES

1. Shaw, G. R. *The genus Pinus*. Pubs. Arnold Arboretum no. 5. Cambridge, Mass. (1914).
2. Erdtman, H., *Svensk Kem. Tid.* 56 (1944) 95.
3. Lindstedt, G. *Acta Chem. Scand.* 3 (1949) 759.
4. Lindstedt, G. *Ibid.* 3 (1949) 763.
5. Lindstedt, G. *Ibid.* 3 (1949) 755.

Received October 12, 1949.