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Constituents of Pine Heartwood

XIV.* The Heartwood of *Pinus monticola* Dougl.

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Pinus monticola, 'Western White Pine', is endemic to the west coast of North America. It belongs to the section *Haploxylon* and is closely related to *P. strobus*. The heartwood constituents of *P. strobus* and of another *Haploxylon* pine, *P. cembra*, have been investigated by Erdtman [1, 2]. These pines contain a few substances which have also been found in *Diploxylon* pines (pinosylvin monomethyl ether, pinocembrin and pinobanksin), as well as other substances which do not seem to occur in *Diploxylon* pines. Thus, both *P. strobus* and *P. cembra* contain pinitol (monomethyl ether of *d*-inositol), chrysin (5,7-dihydroxyflavone) and tectochrysin (5-hydroxy-7-methoxyflavone). In addition, *P. strobus* was found to contain two new substances, known as pinostrobin (5-hydroxy-7-methoxyflavanone) and strobopinin, which seems to be a C-methyl dihydroxyflavanone. The position of the methyl and the hydroxyl groups has not yet been definitely determined.

The heartwood of *P. monticola* was extracted with ether and acetone in the same way as that described for *P. montana* [3]. The ether extract (2.5 % of the heartwood) took the form of a dark brown syrup which did not crystallise. A phenolic fraction, amounting to 2 % of the extract (or 0.05 % of the heartwood) could be prepared from the latter by the method referred to previously [3]. Since this quantity was large when compared with the amounts of pure phenols isolated from the acetone extract, it was deemed advisable to investigate the ether extract too. The phenolic products (along with some resinous material) were precipitated from the extract with light petroleum, which dissolves the resin acids. The precipitate was then treated in the same way as the acetone extract. The sodium bicarbonate and sodium carbonate fractions yielded no

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crystalline products, but the 0.2 % sodium hydroxide fraction contained a small quantity of chrysin and large amounts of resinous products. Pinosylvin monomethyl ether (about one sixth of the quantity isolated from the acetone extract) and a very small quantity of tectochrysin were isolated from the 4 % sodium hydroxide fraction.

The acetone extract was divided into fractions in the usual manner (see Part IX[3]). The water-soluble part contained pinitol and *l*-arabinose, part of which was isolated by precipitation as *p*-bromophenylhydrazone. This quantity was too small to allow complete purification, but the identity of the sugar appears to be clearly established.

The quantity of 'membrane substances' (0.15 % of the heartwood) was greater than in most pines hitherto investigated, although a comparatively large part of the phenols could be extracted by ether. The sodium carbonate fraction contained a small quantity of chrysin, which was identified by acetylation and mixed melting-point of the acetate. The 0.2 % sodium hydroxide fraction yielded an additional quantity of chrysin together with small amounts of a pale yellow crystalline compound with m. p. 224—226° and $[\alpha]_D^{20}$—66° (pyridine, $c = 1.6$). This compound proved to be identical with strobopinin from *P. strobus*. Erdtman[1] reports m.p. 225—227° and $[\alpha]_D^{20}$—60.5° (methanol, $c = 0.5$). (Pyridine was preferred by the present author on account of the fact that the solubility in methanol is too low to allow accurate determinations when only small amounts of the substance are available.)

The 4 % sodium hydroxide fraction contained tectochrysin (which was precipitated as its sodium salt) and pinosylvin monomethyl ether.

The following yields were obtained from 8.4 kg of air-dried heartwood:

Ether extract	206 g	(2.5 %)	
'Membrane substances'	12.7 g	(0.15 %)	
Pinitol	10.9 g	(0.13 %)	
l-Arabinose about	0.02 g		
Chrysin	0.3 g	(0.004 %)	
Strobopinin	0.2 g	(0.002 %)	
Tectochrysin	0.9 g	(0.01 %)	
Pinosylvin monomethyl ether	3.9 g	(0.05 %)	(0.5 g of which came from the ether extract)
Neutral fraction of acetone extract	10.0 g	(0.12 %)	

The yields of phenolic substances were extremely low, but it is evident that *P. monticola* differs from *Diploxylon* pines in the occurence of pinitol and of flavones (chrysin, tectochrysin). Its close relationship to *P. strobus* is demonstrated by the presence of strobopinin in both these pines. It may be men-

tioned here that strobopinin has also been isolated from the heartwood of *P. Lambertiana*, which is closely related to *P. strobus* and *P. monticola* (will be published in a forthcoming paper). In addition to the typical *Haploxylon* heartwood constituents, *P. monticola* also contains pinosylvin monomethyl ether and *l*-arabinose, which seem to occur in almost all pines. It is true that *l*-arabinose has not been isolated from *P. strobus* and *P. cembra*, but this is apparently due to the fact that no search has been made for it.

EXPERIMENTAL

The wood used for the investigation was supplied by Dr. A. B. Anderson, Portland, Oregon, U.S.A.

The heartwood gave a brick-red colour when stained with diazotised benzidine solution. 8.4 kg of air-dried fine-ground heartwood were extracted with ether for 24 hours and then with acetone for 48 hours. The ether extract (206 g) took the form of a brown syrup, which did not crystallise even after several months. 23 g of this syrup were treated with 200 ml of light petroleum, which dissolved 16 g. The solution was decanted, and the sticky brown residue extracted by boiling water (200 + 300 ml). The aqueous extract was cooled and shaken with ether. The ether solution was evaporated, leaving 0.49 g of a light brown resinous product. As this experiment indicates that the ether extract may contain rather much phenolic products, the whole extract was investigated.

Investigation of the ether extract

The entire remainder of the ether extract (183 g) was treated with 500 ml of light petroleum. The solution was separated from the residue by decanting and the solvent removed by evaporation, leaving 132 g of a light yellow viscous oil. It gave a thick pale yellow precipitate with cyclohexylamine in acetone solution. This reagent is known to form insoluble salts with resin acids [4]. The fraction soluble in light petroleum was not investigated further.

The insoluble residue was dissolved in ether (200 ml) and shaken with saturated sodium bicarbonate, saturated sodium carbonate, 0.2 % sodium hydroxide and 4 % sodium hydroxide solutions (2 × 100 ml of each). The fractions are referred to as EB, EC, EH_1 and EH_2 respectively. Each fraction was acidified and extracted with ether. The ether solutions were dried over anhydrous sodium sulphate and the ether evaporated.

EB yielded a comparatively large quantity of a brown non-crystalline resinous product. It was not investigated further.

EC yielded only a small quantity of a brown non-crystalline solid.

EH_1 was a brown syrup (about 10 g). It did not crystallise, but a small part of it could be extracted by boiling water. The aqueous extract was shaken with ether and the ether evaporated. The yellow sticky residue left a small quantity of an insoluble product when stirred with methanol. This product (about 20 mg) proved to be crude chrysin, m. p. 255—263°. It was combined with the chrysin coming from the acetone extract.

EH_2 was a brown suryp, similar to EH_1. Very little could be extracted from it by boiling water, but when the insoluble residue was vacuum-distilled, the distillate showed

some tendency to crystallise. It was stirred with ether and the insoluble crystalline residue removed by filtration. The filtrate was evaporated and the ether treatment repeated twice. Thus, two low-melting fractions (m. p. about 110°) and one high-melting fraction (m. p. 147 – 152°) were obtained. The low-melting fractions were vacuum-distilled and recrystallised from 50 % acetic acid, yielding 0.5 g of crude pinosylvin monomethyl ether, which was combined with the corresponding fraction from the acetone extract. 20 mg of crude tectochrysin (m. p. 157 – 160°) were isolated from the high-melting fraction after sublimation in a vacuum and recrystallisation from ligroin.

Investigation of the acetone extract

After the acetone extract had been left standing for some days, a small quantity of a colourless crystalline precipitate had formed. It was collected and recrystallised twice from ethanol, yielding 0.2 g of pinitol. It melted at 183 – 186° and gave no m. p. depression with pinitol from *P. Lambertiana*. $[\alpha]_D^{20}$ + 64.7° ± 0.5° (water, c = 5.4). It did not reduce Fehling's solution.

On the removal of the acetone from the extract by distillation, an aqueous solution (= W, about 100 ml) and a brown resinous product remained. They were separated and the resin treated with ether. The ether-insoluble 'membrane substances' were separated by filtration and stirred with 300 ml of cold water to remove pinitol and sugars. This water was then combined with W and washed with a little ether which was combined with the ether solution of the resinous product. The ether solution was divided into fractions in the same way as described for the ether extract. The fractions are referred to as B, C, H_1 and H_2, respectively. The remaining ether solution (neutral fraction) was concentrated to a brown turpentine-smelling oil (10 g). It was not investigated further.

W: The aqueous solution reduced Fehling's solution and gave a faint pentose colour reaction with phloroglucinol and hydrochloric acid. It was evaporated to dryness in a vacuum. The remaining yellowish-brown syrup (16 g) was probably a mixture of pinitol and arabinose. After two recrystallisations from ethanol it yielded 10.7 g of pinitol which was combined with the pinitol found before. The mother liquors were then concentrated to a small volume. On cooling, crystalline precipitates formed which reduced Fehling's solution. Since it seemed almost impossible to separate arabinose from an excess of pinitol by recrystallisation, the separation of the whole mixture was not undertaken, and only 0.3 g of it was precipitated with *p*-bromophenylhydrazine in dilute acetic acid solution. A pale yellow crystalline precipitate soon formed. It was separated and treated with benzaldehyde in 50 % ethanol solution on a water bath for 30 minutes to liberate the sugar again from the hydrazone. The benzaldehyde and its hydrazone were then removed by ether extraction, and the remaining water solution evaporated, yielding an almost colourless syrup. After two recrystallisations from ethanol, a colourless crystalline product was obtained (20 mg), melting at 153 – 155°. $[\alpha]_D^{20}$ + 99° ± 2° (equilibrium rotation in water, c = 1.6). Reported for *l*-arabinose: m. p. 159°, $[\alpha]_D^{20}$ + 105.5°. Since mannose, fucose and arabinose are the only sugars that give precipitates with *p*-bromophenylhydrazine in the cold, the identity of the arabinose is definitely established.

B was a brown viscous oil (2 g). It was not investigated further.

C yielded a brown syrupy product which showed some tendency to crystallise. When it was stirred with a few ml of methanol, a yellowish insoluble product was formed, which

was separated by filtration. Evaporation of the methanol from the filtrate and repeated stirring with methanol yielded an additional quantity of insoluble product. This product was recrystallised from glacial acetic acid, yielding brownish yellow crystals melting at 268—274°. After sublimation in a vacuum and two recrystallisations from acetic acid a yellow crystalline product (0.3 g), m. p. 273—275°, was obtained. (Reported for chrysin: 275°). Part of this quantity (0.15 g) was acetylated with acetic anhydride and a few drops of pyridine. After a few hours, a colourless precipitate was separated from the mixture and was recrystallised twice from ethanol. A colourless fibrous crystalline product was obtained, m. p. 193—194°. It gave no melting-point depression when mixed with an authentic specimen of chrysin diacetate. Erdtman reports m. p. 194—196° for chrysin diacetate [1], but earlier investigators obtained lower values [5].

H_1 was a light brown syrupy product which did not crystallise. It was stirred with a few ml of methanol, and the insoluble residue separated. After one recrystallisation from glacial acetic acid it yielded a small quantity of chrysin, m. p. 274—276°, which was added to the chrysin from C. The filtrate was concentrated to a viscous oil and the methanol treatment repeated. An almost colourless insoluble precipitate, melting at 207—213°, was separated from the solution. It was recrystallised from 50 % acetic acid four times, yielding 0.15 g of pale yellow needles, m. p. 224—226°, no depression of the m. p. when mixed with strobopinin from *P. strobus*. $[\alpha]_D^{20} - 66° \pm 1°$ (pyridine, $c = 1.6$). It was possible to collect about 0.1 g of less pure strobopinin from the mother liquors.

The methanol filtrate was evaporated again, yielding a brown syrup, but no further crystalline products could be obtained from it by treatment with methanol or with ether. Extraction with boiling water yielded only a few mg of chrysin.

H_2: When the ether solution was shaken with 4 % sodium hydroxide, a yellow crystalline precipitate was formed (H_{21}). This was separated from the solution and treated with dilute sulphuric acid, yielding a pale yellow crystalline product. After one recrystallisation from chloroform-ligroin and two from ligroin, yellow crystals (0.8 g), melting at 163—165° were obtained. When acetylated with acetic anhydride-pyridine, this substance yielded a crystalline colourless acetate, m. p. 154—156°. Mixed m. p. with an authentic specimen of tectochrysin acetate 153—155°.

The sodium hydroxide solution was acidified and extracted with ether. The ether solution was dried over anhydrous sodium sulphate and the ether removed by distillation. The residue (H_{22}) was a brown viscous oil, which soon crystallised to a great extent. The crystals (m. p. 115—120°) were collected and distilled in a vacuum. The distillate, which crystallised on cooling, was recrystallised from 50 % acetic acid. Yield, 3.0 g of colourless crystals, m. p. 119—121°, mixed m. p. with pinosylvin monomethyl ether 120—122°, It was possible to collect 0.4 g of less pure product from the mother liquors.

SUMMARY

The heartwood of *Pinus monticola* Dougl. has been investigated. Pinitol, *l*-arabinose, chrysin (5,7-dihydroxyflavone), strobopinin (probably a C-methyl dihydroxyflavanone), tectochrysin (5-hydroxy-7-methoxyflavone) and pinosylvin monomethyl ether were isolated from it.

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