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Constituents of Pine Heartwood

XV.* The Heartwood of *Pinus excelsa* Wall.

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Pinus excelsa, 'Bhutan Pine', is a *Haploxylon* pine growing on the slopes of the Himalayas in northern India. The botanists [1] consider it to belong to the group *Strobi*, containing, among other pines, *P. strobus* and *P. monticola*. The heartwood constituents of these latter two pines have already been investigated (see Parts V[2] and XIV[3]). The present investigation shows that the botanical relationship between *P. excelsa* and the above-mentioned pines is clearly demonstrated by the heartwood constituents.

The heartwood was extracted with ether and acetone as described before [4]. The ether extract (14 % of the heartwood) deposited large crystals after some days. Just as in the case of *P. strobus* and *P. monticola*, even the ether extract had to be investigated. The 0.2 % sodium hydroxide fraction contained small amounts of chrysin and pinobanksin, and the 4 % sodium hydroxide fraction contained tectochrysin and a considerable amount of pinosylvin monomethyl ether (about 40 % of the total yield of this substance).

The acetone extract was fractionated in the usual manner. The water-soluble portion contained pinitol and *l*-arabinose, which were separated by precipitation of the sugar as its *p*-bromophenylhydrazone. The sodium carbonate fraction gave a good yield of pinobanksin, precipitated as its sodium salt. Some chrysin was co-precipitated with the pinobanksin, and more chrysin was found in the filtrate. (Pure chrysin gives no precipitate with sodium carbonate solution.) Pinocembrin could be isolated from the 0.2 % sodium hydroxide fraction. The 4 % sodium hydroxide fraction contained a small quantity of tectochrysin (precipitated as its sodium salt) along with much pinosylvin monomethyl ether.

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The yields obtained from 6.5 kg of air-dry heartwood are tabulated below. Only 392 g of the ether extract (total weight 917 g) were investigated, and from those values the theoretical yields for the entire ether extract have been calculated.

Substance	Found in acetone extract	Found in 392 g of ether extract	Calc. for the entire ether extract	Total yield
'Membrane substances'	8.6 g	—	—	8.6 g (0.13 %)
Pinitol + *l*-arabinose	7.4 g *	—	—	7.4 g (0.11 »)
Chrysin	2.4 g	1.0 g	2.3 g	4.7 g (0.07 »)
Tectochrysin	0.4 g	1.8 g	4.2 g	4.6 g (0.07 »)
Pinobanksin	7.5 g	0.4 g	0.9 g	8.4 g (0.13 »)
Pinocembrin	0.9 g	—	—	0.9 g (0.01 »)
Pinosylvin monomethyl ether	53 g	16 g	38 g	91 g (1.4 »)
Neutral fraction	5.1 g	12 g	28 g	33 g (0.5 »)

The specimen of *P. excelsa* investigated here gave very high yields of phenolic substances. The content of pinosylvin monomethyl ether was of the same magnitude as in the best specimens of *P. sylvestris* hitherto investigated. *P. excelsa* resembles other *Haploxylon* pines in its content of pinitol, chrysin, and tectochrysin, but differs from them in its large content of pinobanksin. This substance has been isolated from several *Diploxylon* pines, but of *Haploxylon* pines, only *P. strobus* yielded a very small quantity of it [2]. Strobopinin, a characteristic heartwood constituent of *P. strobus*, *P. monticola*, and *P. Lambertiana*, could not be found in *P. excelsa*. A comparatively large fraction of the heartwood could be extracted with ether (14 %), which indicates that the wood was rich in resins. The ether also extracted large amounts of phenols, although the wood had a comparatively large content of 'membrane substances'. *P. strobus* behaves like *P. excelsa* in this respect [2], but from *P. monticola* only a small part of the phenols can be extracted by ether [3].

To sum up, the heartwood of *P. excelsa* contains the substances which seem to be characteristic of *Haploxylon* pines (pinitol, chrysin and tectochrysin). It also, however, has some features in common with many *Diploxylon* pines (high yields of pinobanksin, pinosylvin monomethyl ether and ether extract).

* The whole mixture was not separated. Only 1.0 g of pinitol and 0.2 g of *l*-arabinose were isolated in a pure state.

EXPERIMENTAL

The wood used for the investigation came from Wood Technologist Forest Research Institute, Dehra Dun, India. The heartwood gave a strong dark red colour when stained with diazotised benzidine solution. The air-dried, fine-ground heartwood (6.5 kg) was extracted with ether for 24 hours and then with acetone for 48 hours. The extracted wood then gave a somewhat weaker colour reaction with the benzidine reagent, but further extraction did not weaken this colour reaction any more. (Acetone for seven days and benzene, ethanol and ethyl acetate for 24 hours were tried.) It is evident that part of the colour obtained with diazotised benzidine is due to insoluble products in the wood.

The ether extract (917 g) was a dark brown syrup, which deposited large crystals in a few days. 36.7 g of the extract were treated with 200 ml of light petroleum, and the yellowish brown sticky residue was extracted with boiling water (2 × 250 ml). The aqueous extracts were cooled and shaken with ether. The ether solution was dried over anhydrous sodium sulphate and concentrated, leaving 0.57 g of a colourless oil, which deposited small crystals. Since the ether extract seemed to contain comparatively large amounts of phenols, it was necessary to investigate a larger portion of it.

Investigation of the ether extract

Part of the ether extract (392 g) was treated with light petroleum (1 l). The solution was separated from the residue by decantation and the solvent evaporated, leaving 193 g of a pale yellow oil, which deposited large crystals (probably resin acids). It was not investigated any further. The residue was dissolved in ether (700 ml) and the ether solution was shaken with saturated sodium bicarbonate, saturated sodium carbonate, 0.2 % sodium hydroxide (200 + 150 ml of each) and, finally, with 4 % sodium hydroxide solution (6 × 150 ml). The three first fractions were called EB, EC and EH_1. A yellow crystalline precipitate was formed in the 4 % sodium hydroxide solution. It was separated by filtration (EH_{21}). Filtrate = EH_{22}. Each fraction (except EH_{21}) was acidified and extracted with ether. The ether solutions were dried over anhydrous sodium sulphate and concentrated. The remaining 'neutral fraction' was a reddish-brown oil with a strong fluorescence. Yield, 12.0 g.

EB and *EC* yielded small amounts of a brown sticky product, which did not crystallise.

EH_1 yielded a large quantity of a brown oil (about 100 g). After a few days it began to deposit a small amount of crystals. The mixture was treated with ether and the crystals removed by filtration. They were brownish-yellow and melted gradually at 250—257°*. After vacuum-sublimation and recrystallisation from ethanol, pale yellow crystals (m. p. 272—274°) were obtained. Yield, 1.0 g. A small part of this substance was acetylated with acetic anhydride and pyridine. The acetate, when recrystallised from ethanol, formed long, thin, colourless needles melting at 193—195°. The mixed m. p. with chrysin diacetate was 193—195°.

The ether filtrate was concentrated to an oil again and extracted with boiling water several times. The water extracts were cooled, forming milky suspensions which were extracted with ether. The ether solutions were combined and dried over anhydrous sodium sulphate. On evaporation they yielded a crystalline residue which was recrystal-

* All melting points uncorrected.

lised twice from 50 % acetic acid and once from toluene. Thick colourless needles (0.4 g), m. p. 175—176°, were obtained. They gave no m. p. depression when mixed with pinobanksin. The insoluble residue from the water extraction was a brown sticky product which did not crystallise.

EH_{21}: The crystalline sodium salt was acidified with dilute sulphuric acid, and the liberated phenol was washed and dried, sublimated in a vacuum, and recrystallised twice from chloroform-light petroleum. Yield, 1.8 g of yellow crystals, m. p. 163—164°. The acetate, prepared by heating the substance with acetic anhydride and pyridine for half an hour on a water bath, melted at 154—155° after one recrystallisation from ethanol, and gave no m. p. depression with tectochrysin acetate.

EH_{22} was a brown oil which crystallised readily. It was distilled in a vacuum and the crystallised distillate recrystallised three times from 50 % acetic acid and once from chloroform, yielding 16.3 g of an almost colourless crystalline product, melting at 119—121°. A mixture with pinosylvin monomethyl ether melted at the same temperature.

Investigation of the acetone extract

The acetone extract was concentrated on the steam bath. The residue consisted of a brown resinous product and an aqueous solution (= W). They were separated by decantation and the resinous product treated with ether to precipitate 'membrane substances'. The ether filtrate (1 l) was shaken with 300 ml of water which was combined with W, and the ether solution then divided into fractions in the same way as described for the ether extract. The fractions are called B, C, H_1 and H_2, respectively. The 'membrane substances' were stirred with cold water, the suspension filtered and the filtrate added to W. The 'membrane substances' were then dried, yielding 8.6 g of a light brown powder.

The 'neutral fraction', which was left in ether solution after the alkali extractions, yielded 5.1 g of a brown turpentine-smelling oil on evaporation. It showed a strong fluorescence in ultra-violet light.

W was shaken with a little ether which was combined with the main ether solution (before the fractionation). The water solution was then evaporated to dryness, by vacuum distillation, and the remaining brown syrup dissolved in boiling ethanol. When the ethanol solution was evaporated to a small volume and cooled, it deposited a colourless crystalline precipitate which was separated by filtration. Further evaporation of the mother liquor yielded an additional amount of crystalline products. The combined precipitates (7.4 g) melted gradually between 140 and 165°, reduced Fehling's solution, and gave a pentose colour reaction with phloroglucinol-hydrochloric acid. Further recrystallisation yielded no pure products. Pinitol could easily be isolated after precipitation of the sugar as its phenylosazone. The excess of phenylhydrazine was removed by benzaldehyde, and the excess of benzaldehyde extracted with ether. The remaining water solution was concentrated to a syrup, from which pinitol was obtained after recrystallisation from ethanol. 2.1 g of the mixture thus yielded 1.0 g of pinitol. Its m. p. was 181—184° but could be raised to 184—186° by further recrystallisation. $[\alpha]_D^{20}$ + 64.5° ± 0.5° (water, c = 3.2). A small amount of *l*-arabinose (0.2 g) was isolated by precipitation as *p*-bromophenylhydrazone. The free sugar was liberated from it by treatment with benzaldehyde as described for *P. monticola* [3]. The sugar melted at 156—158° and gave no m. p. depression with *l*-arabinose. $[\alpha]_D^{20}$ + 101° ± 1° (equilibrium rotation in water, c = 2.3).

B was acidified and extracted with ether. On evaporation of the solvent, the ether solution yielded a brown amorphous product (2 g). This was not further investigated.

C: The sodium carbonate extract deposited a pale yellow crystalline precipitate (C_1), which was separated by filtration. The filtrate was extracted with ether (ether solution = C_2) and then acidified. A sticky brown precipitate was formed. It was taken up in ether, the ether solution dried over anhydrous sodium sulphate and then concentrated, leaving a brown oil which slowly crystallised (= C_3).

C_1 was stirred with dilute sulphuric acid, yielding a brown sticky solid, which was extracted with ether. The insoluble residue was separated, dried in the air and extracted with ether in a Soxhlet apparatus for 48 hours. Most of it dissolved, leaving a dark brown insoluble residue (6 g). The ether extracts were combined, dried over anhydrous sodium sulphate and concentrated to a small volume. A yellow precipitate was formed and separated by filtration. It melted at 265—273° and consisted of crude chrysin. The ether solution was shaken with a saturated sodium carbonate solution to precipitate the pinobanksin sodium salt again. The precipitate was collected, treated with dilute sulphuric acid, combined with crude pinobanksin from C_3 (see below) and then recrystallised several times from 50 % acetic acid and from toluene. Finally, 7.5 g of pinobanksin, m. p. 176—177°, were obtained. $[\alpha]_D^{20}$ + 14° ± 1° (methanol, c = 4.5). The m. p. was not depressed on admixture of pinobanksin from *P. Banksiana.*

C_2 was concentrated to a small volume. A pale yellow precipitate was formed and collected. It melted at 255—259° and was combined with the chrysin found in C_1. The crude chrysin was purified by vacuum sublimation and recrystallisation from ethanol, yielding 2.4 g of pure chrysin, m. p. 275—276°. Its acetate melted at 193—195° and gave no m. p. depression when mixed with chrysin diacetate.

C_3 was treated with ether, and the brownish insoluble crystals were collected. They were dissolved in ether, and the solution filtered through aluminium oxide, which adsorbed most of the coloured impurities. The filtrate was evaporated, yielding a crystalline residue, which was recrystallised from toluene. A small amount of crude pinobanksin (m. p. 168—172°) was obtained and combined with the corresponding product from C^I.

H_1 was acidified and taken up in ether. The ether solution was dried over anhydrous sodium sulphate. On concentration, it yielded a reddish-brown oil which deposited a small quantity of crystals. It was treated with methanol and ether and the crystals collected. They were purified by recrystallisation from 50 % acetic acid and vacuum sublimation, yielding a small amount of crude chrysin (m. p. 270—275°) which was added to the corresponding fraction from C_1 and C_2.

After removal of the chrysin, the oil was left standing for some days. Additional crystals were formed and collected after treatment with ether. This product (m. p. 190—192°) was recrystallised twice from 50 % acetic acid. Colourless needles, m. p. 193—194°, were obtained. Yield, 0.9 g. $[\alpha]_D^{20}$ −54.0° ± 0.5° (methanol, c = 3.7). The substance melted at the same temperature when mixed with an equal amount of pinocembrin.

H_2: The 4 % sodium hydroxide extract deposited a yellow crystalline precipitate (H_{21}), which was separated by filtration and treated with dilute sulphuric acid to liberate the phenol. It was sublimated in a vacuum and recrystallised from methanol and from chloroform-light petroleum. Yellow crystals (0.4 g), m. p. 165—166°, were obtained. The m. p. was not depressed on admixture of tectochrysin from the ether extract.

The sodium hydroxide solution was acidified and extracted with ether. The ether solution was dried over anhydrous sodium sulphate and the solvent evaporated. The

brownish residue crystallised readily. The crystals were dissolved in ether, the solution decolourised by filtration through aluminium oxide and evaporated. The crystalline residue was recrystallised twice from 50 % acetic acid, yielding 53 g of a pale flesh-coloured crystalline substance. M. p. 118—119°. The m. p. was not depressed on admixture of pinosylvin monomethyl ether.

SUMMARY

The heartwood of *Pinus excelsa* Wall. has been investigated. Pinitol, *l*-arabinose, chrysin, tectochrysin, pinobanksin, pinocembrin, and pinosylvin monomethyl ether were isolated from it.

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