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Constituents of Pine Heartwood

XVII.* The Heartwood of *Pinus aristata* Engelm.

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Pinus aristata, 'Bristle-cone pine', is a *Haploxylon* pine growing at rather high altitudes on the southern Rocky Mountains in western North America. The botanists generally divide the section *Haploxylon* into two subsections, *Cembra* and *Paracembra*[1]. *P. aristata* is the first pine from the last-mentioned subsection, which has been investigated with regard to its heartwood constituents.

The ether extract of *P. aristata* heartwood (7.7 %) contained comparatively large quantities of phenolic substances, above all pinosylvin monomethyl ether but also the two flavones chrysin and tectochrysin, which have been isolated from all *Haploxylon* pines hitherto investigated. The acetone extract was divided into fractions in the usual way[2]. Small quantities of chrysin and pinocembrin were found in the 0.2 % sodium hydroxide fraction. This pinocembrin was partially racemised, $[\alpha]_D^{20}$—35° (in methanol). The pinocembrin isolated from other pines has always had a specific rotation of about —55°[2]. Since the isolation of pinocembrin has been carried out by similar methods in all cases, it seems probable that this substance has become racemised in the heartwood of *P. aristata* for some unknown reason.

The 4 % sodium hydroxide fraction of the acetone extract yielded tectochrysin (precipitated as its sodium salt) and a large amount of pinosylvin monomethyl ether.

The water-soluble part of the acetone extract consisted mainly of *l*-arabinose, which could be separated by precipitation with *p*-bromophenylhydrazine. The filtrate from this precipitation yielded only a very small quantity of a brown syrup, from which no crystalline products could be isolated. *P. aristata* thus differs from all *Haploxylon* pines hitherto investigated, which

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contain pinitol along with *l*-arabinose. There are, however, some indications of the presence of very small quantities of pinitol even in *P. aristata*. As no specific reaction for pinitol was available, the methoxyl content of the crystalline sugar fraction and of the syrup left after the precipitation of arabinose by *p*-bromophenylhydrazine was determined. The former fraction contained 0.24 % OCH_3, and the latter 5.23 %. (Pure pinitol has 16.0 % OCH_3). These values show that some methoxyl-containing substance is heavily concentrated in the latter fraction. If that substance were assumed to be pinitol, the methoxyl values would correspond to a pinitol content of 1.5 % and 33 % respectively. Thus, the question of whether or not there is any pinitol in *P. aristata* cannot be definitely answered as yet. It would be of great interest to investigate other pines from the subsection *Paracembra* to see if pinitol is a characteristic heartwood constituent of the whole section *Haploxylon* or only of the subsection *Cembra*.

The total yields from 3.7 kg of air-dried heartwood were:

Substance	From the ether extract	From the acetone extract	Total yield
'Membrane substances'	—	4.0 g	4.0 g (0.11 %)
l-Arabinose	—	5.8 g	5.8 g (0.16 »)
Chrysin	4.7 g	0.5 g	5.2 g (0.14 »)
Pinocembrin	—	0.3 g	0.3 g (0.01 »)
Tectochrysin	3.0 g	2.3 g	5.3 g (0.14 »)
Pinosylvin monomethyl ether	11.3 g	25.5 g	36.8 g (1.0 »)
Neutral fraction	9.8 g	10.9 g	20.7 g (0.56 »)

Total weight of ether extract 287 g (7.7 %).

The specimen of *P. aristata* investigated here contained as much pinosylvin monomethyl ether as a rather good specimen of *P. sylvestris*. The yields of chrysin and tectochrysin were also high compared to other *Haploxylon* pines, but the content of pinocembrin was low. Pinosylvin, pinobanksin and strobopinin have not been isolated.

EXPERIMENTAL

The wood used for the investigation had grown at 3500 m altitude on the Rocky Mountains in California, U.S.A. The heartwood gave a dark red colour when stained with diazotised benzidine solution.

The air-dried, fine-ground heartwood (3.7 kg) was extracted with ether for 24 hours and then with acetone for 60 hours. Upon concentration, the ether extract deposited a yellowish brown precipitate, which was separated. It consisted of crude chrysin (m. p. 265°) and was combined with the chrysin found later on in the 0.2 % sodium hydroxide

fraction of the ether extract. The ether was then completely evaporated on the steam bath, yielding 287 g of a brown syrup. 20 g of this syrup were treated with 250 ml of light petroleum and the insoluble sticky residue separated from the solution. The residue was extracted three times with 200 ml of boiling water and the extracts cooled and extracted with ether. The ether solution was dried and concentrated, leaving 0.6 g of a brown semi-crystalline syrup. This quantity corresponds to 0.2 % of the wood, and it may be concluded that the content of phenols in the ether extract is large enough to necessitate a thorough examination of it.

Investigation of the ether extract

The ether extract was treated with light petroleum (1.5 l) and the insoluble residue dissolved in ether (1 l). The light-petroleum solution was concentrated to a yellow syrup (149 g) which deposited crystals, probably resin acids. This fraction was not further investigated.

The ether solution was divided into fractions by shaking with saturated sodium bicarbonate (3 × 100 ml, extract = EB), saturated sodium carbonate (3 × 100 ml, extract = EC), 0.2 % sodium hydroxide (3 × 200 ml, extract = EH_1) and 4 % sodium hydroxide (2 × 200 ml, extract = EH_2). The remaining ether solution, containing neutral substances, was concentrated to a brown oil with a strong fluorescence (9.8 g). The different fractions were acidified and taken up in ether, and the ether solutions dried over anhydrous sodium sulphate and then concentrated on the steam bath.

EB and *EC* yielded small amounts of brown oils, which did not crystallise.

EH_1 deposited yellowish-brown crystals of crude chrysin, melting about 260°. They were combined with the crude chrysin already found in the ether extract and purified by vacuum-sublimation and recrystallisation from ethanol. Yield, 4.7 g of chrysin, m. p. 274—276°*. The diacetate melted at 195—197° and gave no m. p. depression when mixed with an authentic specimen of chrysin diacetate.

EH_2: The 4 % sodium hydroxide extract deposited a yellow crystalline precipitate (EH_{21}), which was separated before the solution (EH_{22}) was acidified. EH_{21} was treated with dilute sulphuric acid, and the resulting pale yellow precipitate separated, dried, and then recrystallised from 50 % acetic acid and from chloroform-light petroleum (twice). Yield, 2.2 g of tectochrysin, m. p. 163—165°. 0.8 g of less pure product (m. p. 162—163°) were isolated from the mother liquors.

EH_{22} yielded a brown oil, which partly crystallised. It was distilled in a vacuum, yielding a yellowish brown distillate which crystallised on cooling. After two recrystallisations from 50 % acetic acid and one from chloroform-light petroleum, pure pinosylvin monomethyl ether (11.3 g) was obtained. M. p. 120—121°.

Investigation of the acetone extract

The acetone extract, after some days, deposited colourless crystals, melting gradually at 152—160°. They were soluble in water, and the solution reduced Fehling's solution. These crystals were combined with the W fraction (see below). The acetone was then evaporated on the steam bath, leaving a brown syrup and a small volume of water solution (about 20 ml). This unusually small quantity of water in the acetone extract explains

* All melting points uncorrected.

why the sugar precipitate appeared in the extract before concentration. The water solution (= W) was separated from the syrup, and the latter was treated with ether (1 l) to precipitate 'membrane substances'. These were separated and stirred with cold water (50 ml). The suspension was filtered, and the filtrate combined with W. Weight of 'membrane substances', after water treatment and drying, was 4.0 g.

The ether solution was then divided into fractions in the usual way [2]. The volumes of alkaline reagents employed for the extractions were: Saturated sodium bicarbonate 3 × 300 ml, saturated sodium carbonate 4 × 200 ml, 0.2 % sodium hydroxide 5 × 250 ml, 4 % sodium hydroxide 2 × 350 ml. The fractions are referred to as B, C, H_1, and H_2. The remaining ether solution was concentrated to a reddish-brown fluorescent oil (10.9 g).

W: The aqueous solution was concentrated by vacuum distillation to a syrup which crystallised when treated with ethanol. The crystals were collected and combined with the crystalline precipitate mentioned above, yielding 5.8 g of a colourless crystalline powder, which reduced Fehling's solution and gave a strong pentose reaction with phloroglucinol and hydrochloric acid. It contained 0.24 % OCH_3, corresponding to only 1.5 % of pinitol. Part of this fraction (4.0 g) was precipitated with *p*-bromophenylhydrazine in dilute acetic acid solution. The precipitate was collected, and the filtrate shaken with benzaldehyde and then with ether to remove excess *p*-bromophenylhydrazine. The water was then evaporated in a vacuum, leaving a small quantity of a brown syrup (about 0.2 g). No crystalline products could be isolated from this syrup. It gave a faint pentose colour reaction, indicating that all arabinose had not been precipitated. OCH_3 = 5.23 %, corresponding to 33 % of pinitol.

The *p*-bromophenylhydrazone was treated with benzaldehyde to liberate the arabinose again as described for *P. monticola* [3]. 2.7 g of *l*-arabinose, m. p. 158—160°, were obtained. $[\alpha]_D^{20}$ + 107° ± 1° (equilibrium rotation in water, c = 2.8).

B yielded a brown non-crystalline syrup (1 g).

C yielded a small amount of a brown oil, which deposited crystals of crude chrysin. This product was combined with chrysin from H_1.

H_1: The sodium hydroxide extract was acidified and extracted with ether, and the ether solution dried over anhydrous sodium sulphate and then concentrated to a brown oil which soon deposited reddish crystals. The oil was stirred with ether, and the crystals separated by filtration. After vacuum-sublimation and recrystallisation from ethanol they yielded 0.5 g of chrysin, m. p. 275—278°. The ether filtrate was concentrated to a syrup and extracted with boiling water several times. The aqueous extracts were cooled and extracted with ether. On concentration, the ether solution yielded a yellow syrup which deposited an insoluble precipitate when treated with methanol. This product melted at 185—189°. After two recrystallisations from 50 % acetic acid, it yielded 0.25 g of pure pinocembrin, m. p. 194—196°. $[\alpha]_D^{20}$ −35° ± 2° (methanol, c = 2.6). Mixed m. p. with pinocembrin from *P. Banksiana* 194—195°.

H_2: The sodium hydroxide solution deposited a crystalline yellow precipitate (= H_{21}), which was collected and treated with dilute sulphuric acid. The resulting pale yellow precipitate was dried and recrystallised from 60 % acetic acid and then from chloroform-light petroleum, yielding 2.3 g of yellow crystals, m. p. 165—167°. It gave no m. p. depression with tectochrysin. The acetate melted at 154—156° alone and on admixture of tectochrysin acetate.

After separation of H_{21}, the 4 % sodium hydroxide solution (H_{22}) was acidified and extracted with ether. On concentration, this extract yielded a large quantity of a brown

oil, which soon crystallised. Crystals of m. p. 114—118° could be separated by filtration, and the filtrate was distilled in a vacuum, yielding a distillate which crystallised on cooling. Both fractions were recrystallised from chloroform. Almost colourless crystals (25.5 g) were obtained. M. p. 119—121°, no m. p. depression with pinosylvin monomethyl ether.

SUMMARY

The heartwood of *Pinus aristata* Engelm. has been investigated. *l*-Arabinose, chrysin, tectochrysin, pinocembrin and pinosylvin monomethyl ether were isolated from it. Pinitol could not be isolated, but the presence of small quantities of a methoxyl-containing substance in the sugar fraction was demonstrated by analysis.

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