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Constituents of Pine Heartwood

XXII.*) The Isolation of Pinostrobin and 3,5-Dihydroxy-7-methoxyflavanone from the Heartwood of *Pinus clausa* Vasey

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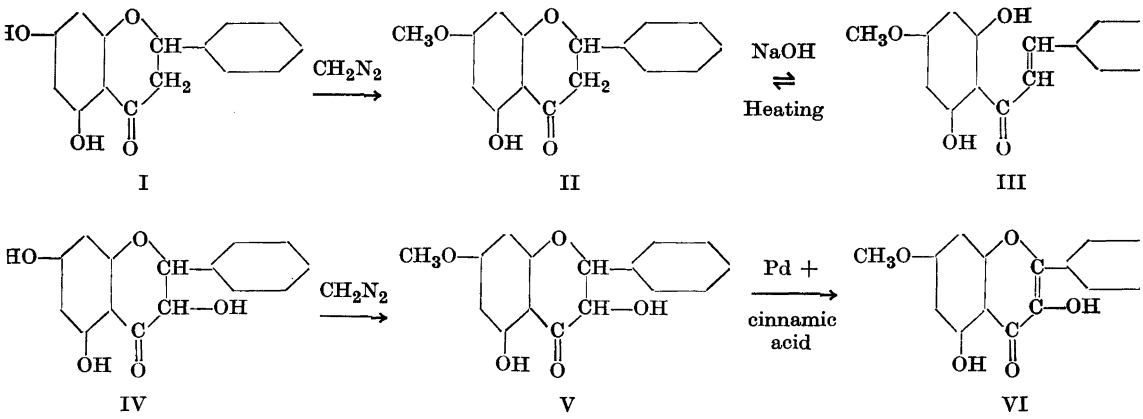
In an earlier paper, the usefulness of paper partition chromatography for analysing pine heartwood extracts was demonstrated [1]. This method has now been applied to a great number of pine species, and a detailed account of this work will appear later. The heartwood extracts from pines belonging to the section *Diploxylon* (Hard Pines) usually contain four phenolic substances, pinosylvin and its monomethyl ether, pinobanksin, and pinocembrin. The acetone extract from one sample of *Pinus clausa*, however, gave a paper chromatogram which contained two additional spots with R_F 0.84 and 0.93 respectively. (The standard solvent and benzidine spraying reagent described in Part XX[1] were used.) The colour and position of one of these spots (R_F 0.93) coincide with that of pinostrobin (5-hydroxy-7-methoxyflavanone), previously isolated from the heartwood of *P. strobus* [2], but the second spot must evidently be due to some new constituent.

In order to isolate the two substances, a large sample of heartwood was extracted with ether and then with acetone, and the acetone extract divided into fractions in the usual manner [3]. Each fraction was analysed by the aid of paper chromatography. The 4 % sodium hydroxide extracts contained the main part of the two new compounds. This extract deposited a colourless sodium salt, from which a crystalline product, melting at 112—113°** was isolated after acidification and recrystallisation. It was optically active, $[\alpha]_D^{20}$ —56° (chloroform). This compound proved to be identical with pinocembrin monomethyl ether (II), which is obtained by treatment of this flavan-

* XXI. *Acta Chem. Scand.* 4 (1950) 772.

** All melting points uncorrected.



I II III

IV V VI

one (I) with diazomethane. The pinostrobin isolated by Erdtman [2] melted at 90—91°. He identified the compound as pinocembrin monomethyl ether by mixed m. p. determination. The difference in melting points may perhaps be due to dimorphism. Due to lack of material Erdtman could not determine the specific rotation of his pinostrobin, but his pinocembrin monomethyl ether had $[\alpha]_D^{20}$—52.7° (chloroform) which is in good agreement with the figure obtained by the present author. The m. p. of racemic 5-hydroxy-7-methoxyflavanone is 101°[4].

Pinostrobin, m. p. 109—112°, has since been isolated from the heartwood of *P. strobus* and of *P. Lambertiana* (not published).

The second unknown substance (R_F 0.84) was isolated from the liquid part of the strong alkali extract, along with additional amounts of pinostrobin. It crystallises from ethanol in colourless needles, m. p. 179—181°, $[\alpha]_D^{20}$—20° (chloroform). The analyses agreed with the composition $C_{15}H_{11}O_4(OCH_3)$, and the compound proved to be identical with pinobanksin 7-methyl ether (V), which has previously been synthesised from pinobanksin (IV)[5]. An independent proof of this structure was furnished by catalytic dehydrogenation to izalpinin (VI = galangin 7-methyl ether) with palladium in the presence of cinnamic acid. Pinobanksin and its 5,7-dimethyl ether can also be converted into the corresponding galangin derivatives by the same method [5].

A very small quantity of a third compound was also isolated from subsequent extracts with 8 and 20 % sodium hydroxide. This compound crystallises in orange red needles, m. p. 152—154°. When dried in a vacuum at 100°, it rearranged to racemic 5-hydroxy-7-methoxyflavanone. Hence, the red compound was apparently 2′,6′-dihydroxy-4′-methoxychalkone (III), formed from pinostrobin under the influence of strong alkali. The presence of

two hydroxyl groups in *o*-position to the carbonyl may facilitate its rearrangement to the flavanone.

When some other heartwood samples from *P. clausa* were investigated, no traces of pinostrobin or pinobanksin monomethyl ether could be found *, nor have these two compounds been observed in any other pine from the section *Diploxylon*. (33 such species have now been investigated.) In *Haploxylon* pines, however, pinostrobin is a common heartwood constituent.

EXPERIMENTAL

The sample of wood came from a pine which had grown at Lake City Branch Station, Florida, USA.

3.2 kg of air-dry heartwood were extracted with ether for 24 hours and then with acetone for 48 hours. The ether extract was concentrated to a dark brown syrup (167 g = 5.5 % of the heartwood). A paper chromatogram of this extract did not give any visible spots on spraying with benzidine reagent. The ether extract was not further investigated.

A paper chromatogram of the acetone extract showed six spots, four of which were due to pinobanksin, pinocembrin (very strong spots), pinosylvin and its monomethyl ether (very weak spots). The two other spots were orange red and developed rather slowly (5—10 minutes after spraying). Their R_F values were approximately 0.84 and 0.93. (See Part XX[1] for the technique of the paper chromatography.) The extract was concentrated to a brown syrup and an aqueous solution containing *l*-arabinose. This solution was decanted off, and "membrane substances" precipitated by addition of ether. The ether solution (700 ml) was shaken with saturated sodium bicarbonate, saturated sodium carbonate, 0.2 % sodium hydroxide, 4 % sodium hydroxide and 8 % sodium hydroxide (3 × 200 ml of each), and, finally, with 20 % sodium hydroxide (200 ml). A colourless, jelly-like precipitate was deposited in the 4 % and stronger sodium hydroxide fractions. The sodium carbonate fraction contained a yellow crystalline precipitate (pinobanksin sodium salt). Each fraction was investigated by paper chromatography. The two new substances accumulated in the 4—20 % alkali fractions, which were further investigated.

Isolation of pinostrobin

All jelly-like precipitates were separated by filtration, combined, and acidified with dilute sulphuric acid, and the suspension extracted with ether. The ether solution was dried over anhydrous sodium sulphate and concentrated, leaving a crystalline residue. After three recrystallisations from methanol, colourless crystals, m. p. 112—113° were obtained. $[\alpha]_D^{20} -56° \pm 2°$ (chloroform, $c = 2.1$). Yield, 0.75 g. A mixture with pinostrobin from *P. Lambertiana* (m. p. 109—112°) melted at 109—111°.

$C_{15}H_{11}O_3(OCH_3)$ (270.3)	Calc.	C 71.1	H 5.22	OCH_3 11.5
	Found	» 71.0	» 5.28	» 11.5

* The identification of the wood samples was carried out in the USA and thus was completely outside the author's control. A confusion with some other species is of course possible, but is rather unlikely, since all the species growing in the south-eastern part of the USA have now been investigated without finding any traces of either compound.

Methylation of pinocembrin

An ether solution, containing about 0.18 g of diazomethane and pinocembrin from *P. Banksiana* (0.7 g, m. p. 194—195°, $[\alpha]_D^{20}$ —54°) was left overnight. On evaporation of the ether, this solution left a colourless crystalline residue, which was recrystallised twice from methanol. Colourless leaflets, m.p. 110—111°, (0.56 g) were obtained. $[\alpha]_D^{20}$ —53°±1° (chloroform, c = 3.9). The m. p. was not depressed on admixture with pinostrobin from *P. clausa* or from *P. Lambertiana*.

Isolation of 3,5-dihydroxy-7-methoxy-flavanone

The filtrate from the 4 % alkali extract was acidified and extracted with ether. The ether solution was dried over anhydrous sodium sulphate and evaporated to dryness, leaving a crystalline residue, which was washed with a few ml of ether. The ether-insoluble part melted at 163—169° and the soluble part (after evaporation of the ether) at 111—112°. This fraction consisted of pinostrobin.

The substance melting at 163—169° (0.85 g) was recrystallised twice from methanol, yielding pale yellow needles, m. p. 170—174°. Coloured impurities were removed by filtration of a solution of the substance in ether through aluminium oxide. The ether was then evaporated and the residue recrystallised from benzene and, finally, from dilute ethanol. Colourless needles, m. p. 179—181°. $[\alpha]_D^{20}$ —20° ± 1° (chloroform, c = 2.7). These data lie very close to those of pinobanksin monomethyl ether (m. p. 180—182°, $[\alpha]_D^{20}$ — 19°), and the m. p. was not depressed on admixture with that substance. The two substances also gave identical spots on the paper chromatogram (R_F 0.84).

$C_{15}H_{11}O_4(OCH_3)$ (286.3)	Calc.	C 67.1	H 4.93	OCH_3 10.8
	Found	» 67.0	» 4.80	» 10.9

Dehydrogenation to izalpinin

The flavanone (0.25 g), cinnamic acid (0.6 g), palladium-charcoal catalyst [6] (0.15 g) and water (15 ml) were reacted in a stainless-steel bomb, rotating in an oil bath at 180° for 80 minutes. The reaction mixture was extracted with ether, and the ether washed with saturated sodium bicarbonate solution three times to remove cinnamic acid. It was then dried over anhydrous sodium sulphate and filtered through aluminium oxide, which adsorbed brownish impurities. The filtrate was concentrated to a yellow crystalline product, which was recrystallised from ethanol. Yellow needles, m. p. 194—195°, were obtained. Yield, 0.08 g. The m. p. was unchanged on admixture with an authentic sample of izalpinin *.

Isolation of 2',6'-dihydroxy-4'-methoxychalkone

The 8 and 20 % alkali extracts were filtered, and the precipitates combined with the precipitate from the 4 % alkali extract (see above). The filtrates were combined, acidified, and extracted with ether. On concentration, the ether extract yielded a yellow syrup, which deposited colourless crystals. These crystals were isolated after trituration with a

* This sample was kindly supplied by Prof. T. R. Seshadri, Delhi, India.

little methanol, and a paper chromatogram of the crystals revealed the presence of pinostrobin and pinosylvin monomethyl ether.

The methanol-soluble portion was concentrated to a syrup. From this, red crystals slowly deposited. The crystals were separated and recrystallised several times from methanol, benzene and from chloroform-light petroleum. Orange red crystals, m. p. 146—147°, were obtained. Further recrystallisation from methanol-water raised the m. p. to 152—154°. Yield, 30 mg. The substance gives a reddish-brown colour with ferric chloride in ethanol solution. It travels a little faster than pinocembrin on the paper chromatogram ($R_F \sim 0.5$). The spot is clearly visible in ultraviolet light by its brownish-violet fluorescence. After spraying with benzidine reagent, it acquires a reddish-brown colour, developing immediately, just as in the case of pinosylvin derivatives.

The red crystals were dried for analysis at 100° in a vacuum for 45 hours. During this time, they transformed into a pale yellow syrup, which crystallised when the tube was scratched with a spatula. The colourless crystalline mass was recrystallised from methanol-water, yielding colourless crystals, m. p. 99—100° alone or on admixture with racemic 5-hydroxy-7-methoxyflavanone [4].

SUMMARY

From one sample of heartwood from *Pinus clausa* Vasey, pinostrobin (5-hydroxy-7-methoxyflavanone) and a new substance have been isolated. The latter substance is identical with pinobanksin 7- methyl ether (3,5-dihydroxy-7-methoxyflavanone) and can be dehydrogenated to izalpinin (3,5-dihydroxy-7-methoxyflavone). The two substances were detected by the aid of paper chromatography.

Other samples of *Pinus clausa* did not contain these substances.

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