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WOOD CONSTITUENTS OF *DUCAMPOPINUS KREMPFII* (LECOMTE) CHEVALIER (*PINUS KREMPFII* LECOMTE)*

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Abstract—*Pinus krempfii* Lecomte is a remarkable pine which on anatomical grounds has been considered to belong to the subgenus *Haploxylon* of the pines in spite of the fact that it has leaves in pairs. Some botanists prefer to regard *P. krempfii* to constitute a subgenus or even a genus of its own, "*Ducampopinus*". A chemical investigation of the wood of "*Ducampopinus krempfii* (Lecomte) Chevalier" very clearly shows that this species is chemically closely related to the *Haploxylon* pines and not to those of *Diploxylon*. Like some pines of the sections and subsections of *Haploxylon* the heartwood contains a series of carbon-methylated flavanones among which demethoxymatteucinol is particularly interesting because it contains two C—CH_3-groups and has not been encountered earlier in any pine heartwood.

THE genus *Pinus* has been extensively investigated from a chemotaxonomic point of view in this laboratory (see previous papers in this series*). Attention was particularly devoted to the phenolic heartwood constituents.[1] The volatile constituents, especially the monoterpenes of the oleoresins of pines, have been studied by Mirov.[2]

On morphological grounds the large genus *Pinus*, comprising some hundred species has been divided into two subgenera, *Diploxylon* and *Haploxylon*. The former have adult leaves borne in clusters of mostly two or three but sometimes five, and the latter in clusters of five or occasionally one to four.

The *Diploxylon* pines were found to contain a characteristic, relatively simple pattern of phenolic heartwood constituents consisting of pinosylvin (3,5-dihydroxystilbene) and its methyl ethers, the flavanone derivatives pinocembrin (2,3-dihydrochrysin) and pinobanksin (2,3-dihydrogalangin). The *Haploxylon* pines contain the same compounds and in addition dihydropinosylvin and flavone derivatives such as chrysin and tectochrysin. Some groups of *Haploxylon* pines, e.g. those belonging to subsection *Strobi* of the section *Cembra* and subsection *Gerardianae* of the section *Paracembra* (according to Shaw's classification[3]) were found to contain carbon-methylated flavonoids, e.g. strobopinin and cryptostrobin (6- and 8-methylpinocembrin, respectively) as well as strobobanksin (6-methylpinobanksin) and strobochrysin (6-methylchrysin). These results indicate that it is possible to distinguish between pines belonging to *Diploxylon* and *Haploxylon* by a chemical investigation of their patterns of phenolic heartwood constituents.

Pinus krempfii Lecomte[4] differs to such an extent from other pines that some botanists

* This is Part 35 in the series "The Chemistry of the Order Pinales". Part 34 appeared in *Acta Chem. Scand.* **18**, 572 (1964).

[1] H. ERDTMAN, *Pure Appl. Chem.* **6**, 679 (1963) and references cited therein. See also R. HEGNAUER, *Chemotaxonomie der Pflanzen* Vol. 1. Birkhäuser, Basel (1962).

[2] N. T. MIROV, *U.S. Dep. Agr. Tech. Bull.* 1239 (1961) and references cited therein.

[3] G. R. SHAW, *The Genus Pinus* (Publ. Arnold Arboretum No. 5). Cambridge, Mass. (1914). Cf. also W. DALLIMORE and A. B. JACKSON, *A Handbook of Coniferae* (3rd Ed.). Arnold, London (1948).

[4] H. LECOMTE, *Bull. Musée, Paris* **9**, 191 (1921).

regard it to constitute a monotypic genus of its own, *Ducampopinus*.[5-7] This remarkable pine occurs in the Nhatrang mountains of Annam. It has smooth bark and fairly broad leaves (up to 5 mm) *in pairs*. The distribution of the stomata of the needles is somewhat unusual as are their resin canals. Florin[8] nevertheless considered *P. krempfii* to belong to the subgenus *Haploxylon*.

It appeared to be of considerable interest to investigate chemically the heartwood of this strange pine in order to find out whether the pattern of constituents agrees with the normal patterns of *Diploxylon* or *Haploxylon* or whether it differs from both of these subgenera. Through the good offices of Dr. Tai-Cong-Tung, Saigon, we have now been able to investigate a small amount of the wood of *P. krempfii*. However, owing to the greatly improved analytical techniques now available a fairly detailed study of the phenolic as well as the diterpene acids could be carried out. Sapwood and heartwood were separately investigated.

The results are summarized in the Tables 1 (phenols) and 2 (resin acids). From the heartwood, pinobanksin, strobopinin, cryptostrobin, demethoxymatteucinol, chrysin and tectochrysin could be isolated in a pure form. Of these compounds demethoxymatteucinol (6,8-dimethylpinocembrin)[9] is the most interesting since it has not before been isolated from any pine heartwood. Its occurrence was not entirely unexpected, however, since mono-C-methylated flavonoids have previously been found in pines.

TABLE 1. PHENOLIC WOOD CONSTITUENTS OF *Pinus krempfii* *

Compound		Ether-insoluble fraction (*A*)	$NaCO_3$-soluble fraction (*B*)	NaOH-soluble fraction (*C*)
Pinosylvin	S	–	–	–
	H	–	–	+
Pinosylvin monomethylether	S	–	–	–
	H	–	–	+
Chrysin	S	–	–	+
	H	+		
Tectochrysin	S	–	–	–
	H	–	–	
Pinobanksin	S	–	–	–
	H	–	+	+(?)
Pinocembrin	S	–	–	–
	H	–	–	–
Pinostrobin	S	–	–	+
	H	+	–	
Strobopinin	S	–	–	+
	H	+	–	
Cryptostrobin	S	–	–	–
	H	+	+	
Demethoxymatteucinol	S	–	–	+
	H	–	+	

* S, sapwood; H, heartwood; –, not present in detectable amounts; (?), identification uncertain; +, identified by TLC; ', isolated.

[5] A. CHEVALIER, *Rev. Botan. Appl. Agr. Trop.* **24**, 7 (1944).
[6] J. T. BUCHHOLZ, *Am. J. Bot.* **38**, 4 (1951).
[7] Y. DE FERRÉ, *Compt. Rend.* **236**, 226 (1953).
[8] R. FLORIN, *Kgl. Svenska Vetenskapsakad. Handl.* Tredje ser., **10** (1), 339 (1931).
[9] S. FUJISE and A. NAGASAKI, *Ber. Deut. Chem. Ges.* **69**, 1893 (1936), and S. FUJISE and T. KUBOTA, *Ber. Deut. Chem. Ges.* **67**, 1905 (1934).

TABLE 2. RELATIVE AMOUNTS OF VARIOUS RESIN ACIDS IN THE WOOD OF *Pinus krempfii*

Acid	Percentage of total	
	Sapwood	Heartwood
Sandaracopimaric	2	1
Pimaric	—*	—*
Isopimaric	—*	—*
Levopimaric + Palustic	25	7
Dehydroabietic	63	35
Abietic	9	57
Neoabietic	present (<0·5)	present (<0·5)

* Could not be detected.

Pinocembrin, pinobanksin, pinosylvin, pinosylvin monomethylether could be detected by chromatographic methods. There are indications that dihydropinosylvin is also present in the wood.

These results clearly demonstrate that *P. krempfii* is chemically closely related to the *Haploxylon* pines and differs distinctly from the *Diploxylon* pines.

We have, of course, no reason to express any opinion about the justification of raising *P. krempfii* to the rank of a subgenus or genus *Ducampopinus*. This is a matter for the botanical taxonomists. We are, however, pleased to find that the chemical method has been found to be of great value for the characterization also of this unusual pine.

EXPERIMENTAL

Chromatography

Thin-layer chromatographic (TLC) examinations of phenolic materials were carried out on silica gel G impregnated with 1·5% sodium ethylenediaminetetraacetic acid (EDTA) using methanol–chloroform (3:100) as solvent. The compounds were detected by observing the fluorescence in u.v. light and by spraying the plates with bis-diazotized benzidine.[10] The

TABLE 3. TLC DATA OF PHENOLIC CONSTITUENTS OF *Pinus krempfii*

Trivial name	Structure	R_f	Colour of spot	
			u.v.	Bis-diazotized benzidine
Pinosylvin	3,5-dihydroxystilbene	0·09	bluish violet	dark violet
Dihydropinosylvin	3,5-dihydroxydibenzyl	0·12	—	red
Pinobanksin	3,5,7-trihydroxyflavanone	0·27	white (luminous)	dark red
Chrysin	5,7-dihydroxyflavone	0·29	brown	red
Pinocembrin	5,7-dihydroxyflavanone	0·40	white (luminous)	violet
Cryptostrobin	8-methyl-5,7-dihydroxyflavanone	0·41	bright brown	orange
Pinosylvin monomethylether	3-hydroxy-5-methoxystilbene	0·43	bluish violet	dark violet
Strobopinin	6-methyl-5,7-dihydroxyflavanone	0·52	brown	orange
Demethoxymatteucinol	6,8-dimethyl-5,7-dihydroxyflavanone	0·58	brown	yellow
Tectochrysin	5-hydroxy-7-methoxyflavone	0·67	white (luminous)	red

[10] G. LINDSTEDT, *Acta Chem. Scand.* **4**, 448 (1950).

compounds appear as round spots and the R_f values vary somewhat with the quality and the dryness of the adsorbent. The R_f values and the colour of the spots are given in Table 3. The impregnation of the silica gel with EDTA was found to improve the separation and reduce tailing of the various compounds.

TLC analyses of the methylated resin acids were carried out on silver nitrate impregnated silica gel.[11]

The gas–liquid chromatographic (GLC) examinations of resin acid methyl esters were carried out on a deactivated silicon rubber (1% E 301) column.[11]

Extraction and Isolation of Components

The sapwood (225 g) and heartwood (280 g) of a small section of the wood of *Pinus krempfii* were separately extracted according to the following procedure.

The ground wood was exhaustively extracted with boiling acetone for 24 hr in a Soxhlet apparatus. The acetone solution was filtered and concentrated to a brownish syrup. This syrup was extracted with boiling ether (2 × 50 ml) leaving an ether-insoluble part (*A*). The ether solution was extracted with aq. Na_2CO_3 (10%, 3 × 50 ml). The combined aqueous solutions were acidified and worked up in the usual way yielding a carbonate-soluble fraction (*B*). The ether solution was then extracted with aq. NaOH (5%, 3 × 50 ml). The combined alkaline fractions were acidified yielding the alkali-soluble fraction (*C*). The extracted ether solution was concentrated to a yellowish, oily, neutral fraction (*D*).

1. *Sapwood.* The ether-insoluble fraction (*A*, 0·206 g) was analysed for phenolic compounds by means of TLC. None of the phenolic compounds listed in Table 1, were detected.

The sodium carbonate-soluble fraction (*B*, 0·285 g) was analysed for phenolic compounds by means of TLC (see Table 1) and after esterification with ethereal diazomethane for resin acid methyl esters by means of TLC and GLC (see Table 2). The relative amounts of the various resin acid esters were estimated from the areas of the GLC peaks.

The sodium hydroxide-soluble fraction (*C*, 0·051 g) was analysed for phenolic compounds by means of TLC. The results are given in Table 1.

The "neutral" fraction (*D*, 0·394 g) has not been investigated.

2. *Heartwood.* A TLC analysis of the ether-insoluble fraction (*A*, 1·134 g) indicated the presence of small amounts of phenolic compounds (see Table 1).

The sodium carbonate fraction (*B*, 1·719 g) contained both phenolic compounds and resin acids. In order to get an approximate estimation of the relative amounts of these two types of compounds a small amount of the fraction (0·019 g) in pyridine (0·2 ml) was treated with hexamethyldisilazane (0·1 ml) according to the method of Waiss *et al.*[12] The silylethers and silylesters thus obtained were analysed by GLC (temp. 190°) under the conditions described in Ref. 11. The peaks were identified by comparison with authentic materials. The relative retention times for the silyl esters of resin acids were shorter than those of the silyl ethers of the phenolic compounds. We hope to discuss the GLC-analysis of silyl derivatives of various wood constituents in more detail in a separate paper.

Fraction *B* was shown to contain about 14·5 per cent of resin acids, 76·2 per cent of phenolic compounds and 9·3 per cent of unknown constituents. The results of separate analyses for phenolic compounds (TLC) and for resin acids (TLC and GLC of a methylated sample) are shown in Tables 1 and 2, respectively.

Sodium hydroxide-soluble fraction (*C*, 2·844 g): When the heartwood extract was treated

[11] T. NORIN and L. WESTFELT, *Acta Chem. Scand.* **17**, 1828 (1963).
[12] A. C. WAISS, JR., R. E. LUNDIN and D. J. STERN, *Tetrahedron Letters* 513 (1964).

with aq. NaOH according to the general procedure described above a yellow crystalline precipitate was deposited which was separated by filtration and then treated with aqueous sulphuric acid (2 N). The product was recrystallized from chloroform–light petroleum (b.p. 40–60°) yielding pure tectochrysin (0·453 g), m.p. 163–164°.*

Part of fraction *C* (1·542 g) in chloroform (5 ml) was adsorbed on silica gel (110 g, previously impregnated with 1·65 g of the disodiumsalt of ethylenediaminetetraacetate, EDTA, and dried at 120° for 12 hr). Elution with the solvents indicated gave the following fractions:

1. Chloroform (Ch.) (425 ml), 0·0 g; 2. Ch. (80 ml), 0·017 g; 3. Ch. (60 ml), 0·275 g; 4. Ch. (30 ml), 0·360 g; 5. Ch. (60 ml), 0·255 g; 6. Ch. (100 ml), 0·050 g; 7. 2% methanol (M.) in Ch. (600 ml), 0·130 g; 8. 5% M. in Ch. (450 ml), 0·200 g; 9. 10% M. in Ch. (600 ml), 0·057 g.

Fraction 1 did not contain any phenolic material as shown by TLC analysis. Fraction 2 was crystalline. Recrystallization from methanol yielded pinostrobin, m.p. 98·5–100°, $[\alpha]_D$ −51° (*c*, 0·59 in $CHCl_3$).

Fraction 3 was crystalline and recrystallization from methanol yielded demethoxymatteucinol, m.p. 206–207°, $[\alpha]_D$ −50° (*c*, 2·18 in acetone) (Lit.[9]: m.p. 202°, $[\alpha]_D$ −50°, in acetone). The compound was easily characterized by its NMR spectrum (recorded on a Varian A-60 instrument; solvent 80% hexadeuterosulphoxide in deuterochloroform). The shifts are given in ppm δ from TMS (internal standard). The spectrum showed the following signals: δ 7·48 (5 H, narrow signal group) due to the aromatic protons of ring B; δ 5·50 (1 H, part of an ABX-spectrum; J_{AX} 5 c/s, J_{BX} 11 c/s) due to the C(2)-proton; δ 3·0 (2 H, the AB part of the ABX spectrum; since the A and B protons have similar chemical shifts the AB coupling constant could not be observed); δ 2·00 (6 H singlet) due to the C(6)- and C(8)-methyl groups.

The mother liquors from the crystallizations of demethoxymatteucinol were analysed by TLC and the following compounds were detected; pinostrobin, demethoxymatteucinol, strobopinin, cryptostrobin, pinosylvin monomethylether and pinocembrin.

Fraction 4 on standing deposited crystals which were recrystallized from aq. acetic acid (50%) yielding strobopinin as yellowish needles, m.p. 224–225°, $[\alpha]_D$ −20° (*c*, 0·80 in MeOH). The mother liquors contained pinostrobin, demethoxymatteucinol, strobopinin, cryptostrobin, pinosylvin monomethylether and pinocembrin.

Fraction 5 on recrystallization from aq. acetic acid (50%) gave cryptostrobin, m.p. 196·5–199·5°, $[\alpha]_D$ −32° (*c*, 1·48 in MeOH). The mother liquors contained strobopinin, cryptostrobin, pinosylvin monomethylether, pinocembrin and chrysin.

A TLC analysis of fraction 6 (a brownish oil) showed the presence of pinosylvin monomethylether, pinocembrin, and chrysin.

Fraction 7 was crystalline. Recrystallization from chloroform yielded chrysin, m.p. 271–273°. The mother liquors were analysed by TLC and the following compounds were detected: pinosylvin monomethylether, pinocembrin, chrysin, pinosylvin and possibly also pinobanksin and dihydropinosylvin.

The oily fractions 8 and 9 were analysed by TLC and chrysin, pinosylvin and possibly also pinobanksin, dihydropinosylvin were detected in the mixture. One further spot with R_f value 0·04 (colour with bisdiazotized benzidine, dark brown) was also detected.

The neutral fraction (*D*, 3·114 g) was analysed for the presence of resin acid methyl esters by TLC and GLC. However no such esters could be detected and the fraction has not been investigated further.

* All compounds isolated (except demethoxymatteucinol) were identified by direct comparisons with authentic samples (mixed m.p., i.r. and u.v.).