

The Chemistry of the Order Cupressales

56.* Heartwood Constituents of *Fitzroya cupressoides* (Molina) Johnston

H. ERDTMAN and K. TSUNO

Department of Organic Chemistry, Royal Institute of Technology, 100 44 Stockholm 70, Sweden

The heartwood of *Fitzroya cupressoides* contains a series of closely related lignans including isotaxiresinol (main component), seco-isolariciresinol, isolariciresinol, and isotaxiresinol 6-methyl ether. The latter is a new lignan. The heartwood of the Japanese yew, *Taxus cuspidata*, contains the same lignans but the neutral constituents of this *Taxus* species are quite different from those of *Fitzroya*. Pinoresinol has been isolated from the resin of *Larix decidua*.

Most genera of *Ordo Cupressales* have now been subjected to at least some chemical investigation.¹ One of the few remaining genera of the family Cupressaceae is *Fitzroya* with the sole species *F. cupressoides* (Molina) Johnston, "alerce". It belongs to the Southern hemisphere subfamily Callitroideae, a very heterogeneous taxon with small, often monotypic, geographically isolated genera.

Fitzroya and the other monotypic genera *Austrocedrus*² and *Pilgerodendron*,³ both formerly included in the genus *Libocedrus s.l.*, are the only species of Cupressaceae in South America. These genera have a similar distribution, *Fitzroya* occurring on and around Chiloe island, Chile, and the adjoining districts of Argentina.

The heartwood of *Austrocedrus* contains the flavanone taxifolin, carvacrol, and β -thujaplicin, that of *Pilgerodendron uviferum* contains sesquiterpenes of cadinane type, e.g. δ -cadinol ("pilgerol") and a phenol of unknown constitution. The heartwood of *Fitzroya* is reddish, has a straight grain and, superficially, resembles the heartwoods of *Sequoia sempervirens* and of *Taxus*.

Extraction with methanol or acetone gave considerable amounts (8-10%) of an essentially phenolic product. The neutral fraction contained phytosterols and a trace of a high melting compound. The main crystalline phenolic constituent (ca. 1% of dry wood) was isotaxiresinol (II c). Minor components were seco-isolariciresinol (I), isolariciresinol (II a) and another

* Part 55: *Chem. Commun. London* 1968 233; Part 54: *Tetrahedron* 1968 3399.

phenol whose composition and spectral properties indicated that it was also a lignan. On methylation it gave isotaxiresinol trimethyl ether (isolariciresinol dimethyl ether) (II b). It gave a green colour reaction with ferric chloride and on acetylation gave a tetraacetate with two methoxyl proton resonances (3.81, 3.59 ppm). This indicated that the compound could be isotaxiresinol 6-methyl ether. (The corresponding values for isolariciresinol dimethyl ether are 3.80, 3.82, 3.86, and 3.55, for collinusin⁴ 3.86 and 3.62 ppm as compared with 3.76 and 3.75 for isolariciresinol and 3.77 ppm/6H/ for its pentaacetate). The structure (II d) was confirmed by oxidation of the phenol with permanganate which afforded metahemipinic acid.

The *Fitzroya* lignans are easily oxidisable compounds and their isolation is considerably facilitated by chromatography on silica gel mixed with sodium pyrosulphite.

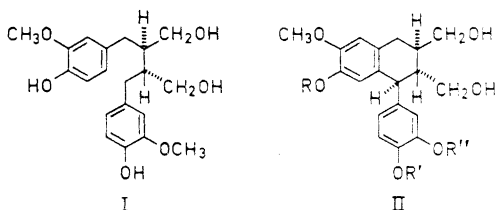
Isotaxiresinol and isolariciresinol readily yield acetonides and these derivatives were isolated from the acetone but not from the methanol extracts. On T.L.C. the acetone extracts gave six distinct spots corresponding to phenolic compounds, the methanol extracts only four.

Thus unlike all other Callitroideae the heartwood of *Fitzroya* contains a mixture of lignans. Several of the Northern Cupressaceae contain lignans.

For comparative purposes, isotaxiresinol was isolated from *Taxus cuspidata*, the Japanese yew, and it was then discovered that this wood also contains the three other *Fitzroya* lignans. The neutral constituents, however, were quite different⁵ from those of *Fitzroya*.

During the isolation of lariciresinol from the resin of *Larix decidua* Miller, it was found that it also contains pinoresinol which was isolated chromatographically. Pinoresinol has formerly been found in the genera *Pinus* and *Picea*.

We are indebted to the late Mr. I. Lagerborg, Talca, Chile, for the sample of *Fitzroya* wood and to Dr. E. W. J. Phillips, Princes Risborough, England, for checking its identity.



I Seco-isolariciresinol

- II a: R = R' = H, R'' = OCH₃: Isolariciresinol
 II b: R = R' = R'' = CH₃: Isotaxiresinol trimethyl ether
 II c: R = R' = R'' = H: Isotaxiresinol
 II d: R = OCH₃, R' = R'' = H: Isotaxiresinol 6-methyl ether

EXPERIMENTAL

Extraction and isolation of phenolic substances.

a) *Extraction with methanol.* Finely ground wood (200 g) was extracted with methanol in a Soxhlet apparatus for 24 h. The solvent was evaporated *in vacuo* to give a reddish-

brown residue (28 g). This was dissolved in methanol and celite (100 g) added. The dried product was then successively extracted with light petroleum (b.p. 40–60°), ether, and chloroform. The petroleum soluble products weighed 0.21 g and the combined ether and chloroform soluble extractives 13.3 g. The latter material was dissolved in chloroform containing 5 % by volume of methanol and the solution chromatographed using the same solvent mixture and a column consisting of silica gel mixed with 2 % sodium pyrosulphite. The latter component is important and, as we have found, is generally useful for the separation of easily oxidisable compounds. The following compounds were successively eluted: 1) seco-isolariciresinol (55 mg or 0.03 % of the dry wood), 2) isolariciresinol (554 mg, 0.28 %), isotaxiresinol 6-methyl ether (130 mg, 0.065 %) and isotaxiresinol (2.3 g, 1.15 %).

Seco-isolariciresinol: This compound, $C_{20}H_{26}O_6$, m.p. 114–116°, $[\alpha]_D^{22} - 30.8$ (*c* 1.0 in acetone), was obtained pure from chloroform containing a little methanol. The mixed melting points with an authentic sample obtained from lariciresinol and with a sample isolated from the wood of *Podocarpus spicatus*¹⁰ kindly provided by Professor L. H. Briggs, showed no depression (114–116°). The IR spectra were superimposable. The dimethyl ether, $C_{22}H_{30}O_6$, m.p. 129–130°, $[\alpha]_D^{22} - 35.7$ (*c* 1.45 in $CHCl_3$) was obtained by methylation with diazomethane. Literature values m.p. from 126.5 to 128.8° and $[\alpha]_D$ from –32.3 to –35.6°.

Seco-isolariciresinol from lariciresinol: Following Ref. 11, (+)-lariciresinol (1.5 g) was dissolved in acetic acid (30 ml) and hydrogenated over palladised charcoal (10 %, 0.9 g). When one mole of hydrogen had been consumed the product obtained after filtration and evaporation of the solution was taken up in $CHCl_3$, shaken with water, dried (Na_2SO_4) and reconcentrated. The residue was crystallised from chloroform containing some methanol. Rods (1.12 g), m.p. 117–119°, $[\alpha]_D - 29.4$ (*c* 1.08 in acetone). Lit.¹⁰ m.p. 112.5–113.5°, $[\alpha]_D^{25} - 35.6^\circ$ (*c* 1.07 in acetone).

The compound was converted into the anhydride,¹¹ $C_{20}H_{24}O_5$, m.p. 121–122°, $[\alpha]_D^{24} - 53.1^\circ$ (*c* 1.1 in acetone), –47.7° (*c* 1.25 in THF). Lit.¹¹ m.p. 116–117°, $[\alpha]_D - 51.8$ (in THF).

Isolariciresinol: The compound, $C_{20}H_{24}O_6$, crystallised from chloroform/methanol. Needles, m.p. 157–158°, $[\alpha]_D^{22} + 69.5^\circ$ (*c* 1.19 in acetone), +43.7° (*c* 1.035 in ethanol). (Lit.¹² m.p. 112°, $[\alpha]_D + 69.4^\circ$, acetone). A sample prepared from lariciresinol when crystallised from aqueous methanol had m.p. 112° but from chloroform/methanol gave needles, m.p. 157–158°. Mixed m.p. showed no depression.

Isolariciresinol tetraacetate: $C_{28}H_{32}O_{10}$, melted at 164–165°, $[\alpha]_D^{22} + 21.3^\circ$ (*c* 1.08 in acetone, $[\alpha]_D^{22} - 3.36^\circ$ (*c* 1.015 in $CHCl_3$)). (Lit.¹² m.p. 162°, $[\alpha]_D + 18.4^\circ$ in acetone).

Isolariciresinol dimethyl ether: $C_{22}H_{28}O_6$, melted at 176–178°, $[\alpha]_D^{25} + 14.8^\circ$ (*c* 1.15 in $CHCl_3$). In the literature^{6,12–15} values varying from 166 to 172° are recorded for the melting point and $[\alpha]_D$ from +15.8 to +20.0°.

Isolariciresinol dimethyl ether anhydride: $C_{22}H_{26}O_5$, m.p. 212–213°, $[[\alpha]_D^{24} + 15.1^\circ$ (*c* 1.05 in acetone), +6.5° (*c* 1.15 in HOAc). Lit.¹² +7.9° (in HOAc).

Isotaxiresinol 6-methyl ether: The compound crystallised from hot water or 2 N acetic acid and was recrystallised from $CHCl_3$:MeOH. Needles, m.p. 181–182°, $[\alpha]_D^{24} + 34.9^\circ$ (*c* 1.49 in acetone). (Found: C 66.5; H 6.8; *m/e* 360. Calc. for $C_{20}H_{24}O_6$: C 66.6, H 6.7; M.w. 360). λ_{max} 214, 384 nm (log ϵ 4.5 and 3.9, respectively). IR: 3480, 3220, 1610, 1510, 1440, 1243, 1220, 1120, 1055, 1000, 940, 870 cm^{-1} . The compound was sparingly soluble in cold water, ether and chloroform, readily soluble in alcohol and acetone. A solution in alcohol gave a green colour reaction on addition of alcoholic ferric chloride.

Isotaxiresinol 6-methyl ether tetraacetate: The phenol was acetylated with acetic anhydride and pyridine at room temperature. From methanol, needles, m.p. 113–114°. $[\alpha]_D^{20} + 8.3^\circ$ (*c* 1.007 in $CHCl_3$). (Found: C 63.4; H 6.0; *m/e* 528. Calc. for $C_{28}H_{32}O_{10}$: C 63.6; H 6.1; M.w. 528). λ_{max} 212, 268 (sh), 275 (sh) and 283 nm (log ϵ 4.6, 3.5, 3.55 and 3.6, respectively). IR: 1764, 1734, 1610, 1520, 1500, 1370, 1250, 1220, 900, 840 cm^{-1} . NMR: Ac.: 2.01, 2.01, 2.19, 2.22 ppm, singlets (each 3H); OMe: 3.59, 3.81 ppm, singlets (each 3H); aromatic protons: 6.22, 7.16 ppm, multiplet (5H).

Isotaxiresinol trimethyl ether: Isotaxiresinol 6-methyl ether in methanol was methylated with a large excess of diazomethane in ether (48 h at room temperature). The compound $C_{22}H_{28}O_6$, crystallised from methanol as needles, m.p. 175–178°, undepressed when mixed with isotaxiresinol trimethyl ether from isotaxiresinol. IR spectra identical.

Permanganate oxidation of isotaxiresinol 6-methyl ether: Isotaxiresinol 6-methyl ether (300 mg) was dissolved in acetone (50 ml). A small amount of sodium carbonate was added, followed (at 70–80°) by powdered potassium permanganate in portions. When decolorisation slowed the solvent was evaporated and the residue was suspended in water and treated with sulphur dioxide. Sulphuric acid was added to pH=2. Continuous extraction with ether gave a gum which was treated with methylamine. The dried product was repeatedly sublimed *in vacuo*. The slightly yellowish needles were finally re-crystallised from methanol. Long needles (2 mg). M.p. and mixed m.p. with authentic metahemipinic acid *N*-methylimide, 160–163°. IR spectra superimposable.

Isotaxiresinol: The compound was easily obtained pure. From 2 N acetic acid, flat rods, m.p. 171¹²,¹² undepressed on admixture with isotaxiresinol isolated from *Taxus baccata* or *T. cuspidata*.

b) *Extraction with acetone*. The concentrated acetone extract was poured into a large volume of ether and the ether soluble material was separated in the usual way into "acidic", "phenolic", and neutral components by extraction with sodium carbonate and alkali. Chromatography of the first two fractions gave the four lignans mentioned above and in addition isotaxiresinol acetonide (amorphous, *m/e* 386) and isolariciresinol acetonide (amorphous, *m/e* 400). The NMR spectra of the acetates of these compounds (amorphous, *m/e* 512, 484, respectively) showed the presence of acetal methyl groups (1.3 ppm, singlet, 6H).

On hydrolysis with hydrochloric acid in methanol these phenols gave isotaxiresinol and isolariciresinol, respectively.

The neutral fraction (0.08 % of the dry wood) was chromatographed on silica gel using successively light petroleum/ether (9/1), light petroleum/chloroform (1/9), and pure chloroform. The second eluate gave "sitosterol", C₂₈H₅₀O, m.p. 139–140°, [α]_D –32° (CHCl₃). Gas chromatography indicated that the product consisted of 16 % campesterol and 84 % β -sitosterol. The third eluate furnished a small amount of a product, needles, m.p. 245–246°, [α]_D²² +1.37° (c 1.09, CHCl₃). (Found: C 79.0; H 11.9; *m/e* 432). The acetate (acetic anhydride/pyridine) melted at 102–105°, [α]_D²² +1.66 (c 1.03, CHCl₃). (Found: C 75.6; H 10.7; *m/e*=474).

Acknowledgement. This work was supported by a grant from *Statens Naturvetenskapliga Forskningsråd* (The Swedish Natural Science Research Council). We thank Dr. B. Thomas for many discussions, for massspectrometric assistance and for improving the English.

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Received November 22, 1968.

Acta Chem. Scand. **23** (1969) No. 6