

## The Chemistry of the Natural Order Cupressales

### XXX\*. Heartwood Constituents of *Juniperus cedrus* L.

JARL RONEBERG

*Organisk-kemiska Institutionen, Kungl. Tekniska Högskolan, Stockholm, Sweden*

The heartwood of *Juniperus cedrus* contains thymoquinone, thujopsene, cuparene, cedrol,  $\delta$ -cadinol, widdrol, "Widdringtonia acid II", nootkatin,  $\beta$ -thujaplicin and carvaerol.

The genus *Juniperus*, which comprises about 60 species, is divided into the sections *Oxycedrus* and *Sabina*. Previous papers<sup>1</sup> have dealt with the wood constituents of a number of junipers, all belonging to the section *Sabina*. In order to compare the two sections chemically a sample of *J. cedrus* (*Oxycedrus*) has now been investigated. The wood was obtained from the Canary Islands.

Only a few papers have been published on the wood oils of *Oxycedrus* junipers. From branches of *J. taxifolia* Shinozaki<sup>2</sup> obtained an oil containing 50 %  $\alpha$ -pinene, an alcohol  $C_{10}H_{18}O$ , an unknown sesquiterpene hydrocarbon and a sesquiterpene alcohol. According to Mousseron<sup>3</sup> a wood pyrolysate of *J. oxycedrus* contained a *d*-cadinene and an *l*-cadinol in addition to a sesquiterpene hydrocarbon and a sesquiterpene alcohol which have not been further investigated. From the wood of *J. communis* Bredenberg<sup>4-6</sup> has isolated ferruginol,  $\Delta^9$ -dehydroferruginol, 9-ketoferruginol and 9,10-diketoferruginol. Cedrene and cedrol have not previously been found in any *Oxycedrus* junipers and Bredenberg<sup>7</sup> has suggested that the presence or absence of cedrene and cedrol can be used to differentiate between the sections *Sabina* and *Oxycedrus*. However, the isolation of cedrol from *J. cedrus* (*Oxycedrus*) described in the present paper makes this suggestion untenable.

In this investigation the wood was extracted with acetone and the acetone extract was evaporated and poured into ten volumes of light petroleum. The light petroleum-soluble oil was separated into neutral and acidic fractions by extraction with alkali. The acidic material afforded the "acid II", m.p. 190.0—191.0°, of *Widdringtonia*<sup>8</sup>. The presence of nootkatin,  $\beta$ -thujaplicin

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and carvacrol in the remaining acidic material was indicated by paper chromatography.

The neutral oil was distilled and the fractions analysed by gas chromatography. According to its infrared spectrum the first fraction, which was yellow, appeared to contain thymoquinone. It also contained hydrocarbons and traces of alcohols. A sample was reduced, and from the product hydrothymoquinone was isolated.

The main component of all the hydrocarbon fractions was thujopsene<sup>9</sup> which was oxidised to the corresponding crystalline aldehyde (widdrenal)<sup>8</sup>. The infrared spectra of these fractions indicated the presence of small amounts of two compounds containing a vinylidene group ( $1645\text{ cm}^{-1}$ ,  $883\text{ cm}^{-1}$ ). One of these hydrocarbons may be identical with the curcumene isomer which appears to be present in corresponding sesquiterpene hydrocarbon fractions of *J. virginiana* and *J. thurifera*<sup>1</sup>. This has not been separated from thujopsene by the gas chromatographic system used.

The question of the presence or absence of  $\alpha$ -cedrene in this wood could not be settled. On gas chromatography several of the hydrocarbon fractions gave a small peak with a retention time very close to that of  $\alpha$ -cedrene but the infrared spectra of these fractions did not show any bands typical of  $\alpha$ -cedrene; this, however, does not exclude the presence of small amounts.

The highest boiling hydrocarbon fraction was ozonised at low temperature and the material unattacked by ozone was identified as cuparene<sup>10</sup>.

Chromatography of the sesquiterpene alcohol fraction gave cedrol,  $\delta$ -cadinol<sup>11</sup> and widdrol<sup>8</sup>. Carbonyl compounds present in the residue, obtained on distillation of the neutral oil, were isolated using the Girard D reagent and chromatographed on alumina. Several liquid fractions were obtained but these have not been further investigated.

The compounds isolated are listed below with very approximate estimates of the amounts present (as percentages of the air-dried wood). Total acetone extract 6.1, light petroleum-soluble acetone extract 5.4, sodium bicarbonate-soluble 0.02, potassium hydroxide-soluble 0.09, neutral 5.3, nootkatin 0.0003,  $\beta$ -thujaplicin (traces), carvacrol 0.01, thymoquinone 0.04, thujopsene 2.2, cuparene 0.1, cedrol 0.06,  $\delta$ -cadinol 0.04, widdrol 0.07, high-boiling carbonyl compounds 0.2.

## EXPERIMENTAL

Rotations were measured in chloroform unless otherwise specified; melting points, taken on a hot stage, are corrected; boiling points are uncorrected. Light petroleum refers to the fraction b.p.  $40-60^\circ$ .

*Isolation of the oils.* The ground, air-dried, heartwood (3.90 kg) was extracted with acetone for 48 h. The acetone was evaporated and the remaining oil poured with stirring into ten volumes of light petroleum. The solution was filtered (precipitate A, 30.1 g) and evaporated. The remaining oil was diluted with two volumes of light petroleum and extracted successively with solutions of sodium bicarbonate (saturated) and potassium hydroxide (2 N). On acidification and extraction with ether the sodium bicarbonate solution afforded a viscous oil B (0.74 g) which was not further investigated. The potassium hydroxide-soluble material C (3.62 g) was isolated in the same way by acidification and extraction with ether. The remaining light petroleum solution was washed with water, dried and evaporated giving neutral material E (205 g).

Table 1. Distillation of neutral fraction E. Total distillate 149 g or 73 %.

Fraction	Weight (g)	B.p./22 mm Hg (°C)	Rotation ( $[\alpha]_D$ )	Refractive index ( $n_D^{25}$ )
Ia	10.0	119–136	–47	1.5018
Ib	19.1	136–137	–78	1.5017
Ic	19.8	137–138	–91	1.5024
Id	16.6	138–139	–87	1.5030
Ie	23.4	139–148	–48	1.5063
If	14.1	148–150	+21	1.5126
Ig	22.5	150–164	+12	1.5131
Ih	14.8	164–166	+ 4	1.5062
Ii	8.7	166–168	+ 5	1.5071
Ik (residue)				

*Alkali-soluble fraction.* The alkali-soluble oil C was dissolved in ten volumes of ether and extracted with a solution of sodium carbonate (2 N). The ether solution on evaporation afforded an oil F (0.98 g). The sodium carbonate solution was acidified, extracted with ether and the ether solution was evaporated yielding an oil G (2.41 g) which partly crystallised on standing. The crystals (32 mg) were filtered off, recrystallised from light petroleum, m.p. 190.0–191.0°,  $[\alpha]_D - 98^\circ$  (c, 2.1) and identified (mixed m.p., IR) as the acid II isolated from *Widdringtonia*<sup>8</sup>. Paper chromatography<sup>13</sup> showed that the non-crystalline part of G contained *nootkatin* and  $\beta$ -*thujaplicin* and the oil F contained *carvacrol*.

*Neutral fraction.* The neutral oil E was distilled through a one metre, vacuum-jacketed, packed column giving the fractions listed in Table 1.

*Gas chromatographic examination of the sesquiterpene hydrocarbon fractions.* Gas chromatograms were run on a Pye Argon Chromatograph as described in a previous paper<sup>13</sup> (temperature 150°, charge 0.025  $\mu$ l).

In Table 2 the approximate areas of individual peaks of different retention times are given as percentages of total peak area.

With an argon flow rate of 25 ml/min the  $R_t$ -values (min) of the following known compounds were:  $\alpha$ -cedrene (prepared from cedrol) 19.1, thujopsene 21.0 and cuparene 46.0.

*Identification of thymoquinone.* The yellow fraction Ia (2.0 g) was suspended in water (5 ml), and the mixture was saturated with sulphur dioxide. The reaction flask was stoppered and allowed to stand for three days. The product was then dissolved in ether, extracted with sodium hydroxide (2 N) and the alkaline solution was acidified and extracted with ether. On evaporation the ether solution gave a crystalline product (0.28 g), which was sublimed in a high vacuum, and identified (mixed m.p., IR) as hydrothymoquinone, m.p. 143.5–144.5°.

Table 2. Gas chromatographic analysis of the sesquiterpene hydrocarbon fractions in Table 1. Argon flow rate 25 ml/min.

Fraction	Peak retention time (min)											
	10.1	12.4	14.6	18.9	21.0	22.9	30.4	33.2	34.9	46.0	48.4	49.9
Ia	1	2	6	6	73						12	
Ib		1	3	9	87							
Ic			1	4	95							
Id					100							
Ie					60	9	9	15	20	5		2
If					1	2	9	42	20	20		6

*Identification of thujopsene.* Fraction Id (1.0 g) in ethanol (95 %, 20 ml) was refluxed with selenium dioxide (0.6 g) for 4 h and the product was filtered and evaporated to dryness. The residue (0.82 g) was recrystallised from light petroleum, m.p. 73.5–74.5°, and identified as widdrenal<sup>8</sup> (mixed m.p. IR).

*Cuparene.* Fraction If (3.0 g) in methylene chloride (15 ml) was ozonised at –70° until an excess of ozone was indicated by the appearance of a blue colour. When the reaction mixture had reached room temperature, hydrogen peroxide (30 %, 8 ml) in potassium hydroxide (10 %, 3 ml) was added dropwise and the mixture was heated on a water bath for 5 min. The organic phase was diluted with a large quantity of ether, separated and evaporated to dryness. The remaining viscous oil was chromatographed on basic alumina (40 g). The first fraction of light petroleum (100 ml) eluted a hydrocarbon (460 mg) which was distilled under reduced pressure. The fraction boiling at 118°/8 mm,  $n_D^{25}$  1.5206,  $[\alpha]_D + 65^\circ$  (c, 2.2) was identified as *cuparene*<sup>10</sup> by comparing its physical constants and infrared spectra with those of an authentic sample.

*Sesquiterpene alcohols.* Fraction Ii (2.52 g) was chromatographed on basic alumina (100 g). Light petroleum eluted an oil (0.22 g), benzene also eluted oily material (0.39 g) (neither of these have been investigated further). Ether-benzene (1:50) eluted a compound (0.26 g) which was recrystallised from ethanol (95 %), m.p. 88.0–88.5°, and identified as *cedrol*. Ether-benzene (3:7) eluted  $\delta$ -*cadinol*<sup>11</sup> (0.17 g) which after recrystallisation from acetonitrile had the m.p. 138.0–139.0°. Ether eluted a compound (0.31 g) which was recrystallised from acetonitrile and sublimed in a high vacuum to yield *widdrol*<sup>8</sup>, m.p. 95.0–96.0°,  $[\alpha]_D + 105^\circ$  (c, 2.2). (All identifications were made by mixed m.p. and IR.) Elution of the column with methanol gave a resinous material.

*High-boiling carbonyl compounds.* A mixture of carbonyl compounds (0.72 g) was separated from a sample of the neutral distillation residue Ik (5.0 g) using Girard D reagent (2.0 g) as described in a previous paper in this series<sup>13</sup>.

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