

The Chemistry of the Natural Order Cupressales

XXXV *. Heartwood Constituents of *Juniperus foetidissima* Willd.

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The heartwood of *Juniperus foetidissima* contains two new sesquiterpene hydroxy acids, α -cedrene, calamenene, a new sesquiterpene oxide and the alcohols cedrol and widdrol. Positive evidence was obtained for the presence of δ -cadinene and an *isocadinene* in addition to several unidentified sesquiterpene hydrocarbons.

In continuation of previous work on the constituents of the genus *Juniperus*¹, the heartwood of *J. foetidissima* Willd.² has been investigated. This species is a tree or shrub 10–12 ft high native to Greece and Asia Minor. The wood used in this investigation had been cut at an altitude of 5 000 ft in the Troodos Forest on the Island of Cyprus.

The heartwood was extracted with acetone and the light petroleum-soluble part of this acetone extract was separated into sodium bicarbonate-, sodium carbonate-, potassium hydroxide-soluble and neutral fractions.

The sodium bicarbonate-soluble material gave two new sesquiterpene hydroxy acids which were separated by chromatography. According to its ultraviolet absorption, hydroxy acid I, m.p. 166–168°, appeared to be an $\alpha,\beta,\gamma,\delta$ -unsaturated acid or the cross-conjugated analogue. Hydroxy acid II, m.p. 152–153° is aromatic with the carboxyl group attached to a benzene ring. The infrared spectra of the two acids are shown in Fig. 1.

Hydroxy acid II was esterified with diazomethane and reduced to a diol which was dehydrogenated with selenium. The main product, according to its infrared spectrum, appeared to be an oxo-compound. Only traces of a compound giving a picrate were obtained.

Most junipers described in this series of investigations have contained carvacrol and tropolones¹ (*cf.* also Ref.³). Neither of these compounds could be detected in this species.

The neutral oil was distilled and the fractions analysed by gas chromatography. The first constant boiling fraction consisted mainly of α -cedrene which was identified as α -cedrenic acid⁴.

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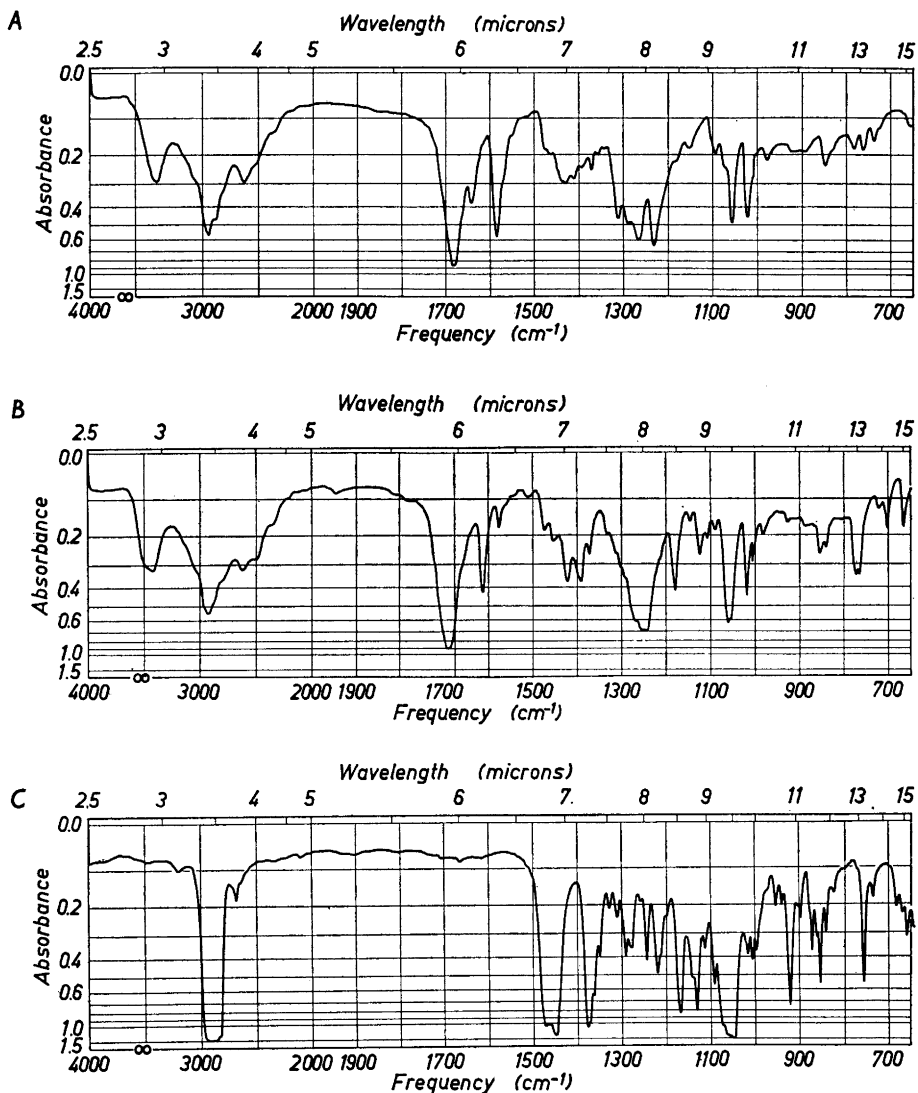


Fig. 1. Infrared spectra of hydroxy acid I, m.p. 166–168° (A), hydroxy acid II, m.p. 152–153° (B) (in KBr) and oxide, b.p. 131°/12 mm (C) (oil).

The slightly higher boiling material that followed gave a low yield of an adduct with maleic anhydride. On chromatography it was separated into three main fractions. The first, on selenium dehydrogenation, gave a good yield of cadalene. The infrared spectrum of this fraction was very similar to that of δ -cadinene⁵ but the ultraviolet spectrum (λ_{\max} 247 $m\mu$) indicated the

presence of yet another compound. δ -Cadinene, isolated from three different sources was shown by Šorm and co-workers to contain an unknown *isocadinene* with two conjugated double bonds⁶. The position of the ultraviolet absorption maximum due to this *isocadinene* (λ_{\max} 246 m μ) agrees well with that of the chromatographic fraction above. This therefore seems to consist of a mixture of δ -cadinene and an *isocadinene* with conjugated double bonds.

The mixture was easily autoxidised and the infrared spectrum of even freshly isolated fractions showed some hydroxyl-absorption. This increased rapidly with time and in a few days the material, although stored in a sealed tube under nitrogen, became a viscous oil.

The next fraction eluted was identified as calamenene^{5,7} by its infrared spectrum and physical constants. On selenium dehydrogenation it gave a good yield of cadalene.

The third fraction afforded an oil, b.p. 131°/12 mm, $[\alpha]_D -69^\circ$. According to elemental analysis, infrared absorption (strong triplet at 1 045—1 075 cm^{-1} , cf. Fig. 1.) and chromatographic behaviour, it appears to be a new sesquiterpene oxide.

Thujopsene, a constituent of most of the junipers investigated in this series¹, could not be detected. Small amounts of thujopsene may, however, occur in this species.

Previous papers in this series describe the heartwood constituents of junipers belonging to the section *Sabina*¹. Several of the fractions containing sesquiterpene alcohols obtained from other members of this section, crystallised immediately. The corresponding fractions from *J. foetidissima* were oils with comparatively low viscosities and none of them crystallised on standing for a year. One of these sesquiterpene alcohol fractions on chromatography gave crystalline cedrol and widdrol⁸.

According to its infrared spectrum, a fraction boiling slightly higher than the sesquiterpene alcohols appeared to contain an α,β -unsaturated oxo-compound.

The distillation residue appeared to contain rather large quantities of a lactone, but this material has not yet been investigated.

The compounds isolated are given below with very approximate estimates of the amounts present (as percentages of the air-dried wood). Total acetone extract 9.9, ether-soluble acetone extract 8.2, light petroleum-soluble acetone extract 6.0, sodium bicarbonate soluble 0.1, potassium hydroxide soluble 1.2, neutral 4.7, hydroxy acid I 0.002, hydroxy acid II 0.008, α -cedrene 0.7, δ -cadinene-*isocadinene* mixture 0.2, calamenene 0.07, sesquiterpene oxide 0.6, cedrol 0.1 and widdrol 0.06.

EXPERIMENTAL

Rotations were measured in chloroform unless otherwise specified; melting points, taken on a hot stage, and boiling points are uncorrected. Light petroleum refers to the fraction b.p. 40—60°.

The air-dried heartwood (10.9 kg) was extracted with acetone for 40 h and fractionated as described in a previous paper in this series⁹. Ether-insoluble acetone extract A (182 g), ether-soluble but light petroleum-insoluble acetone extract B (238 g), light petroleum- and sodium bicarbonate-soluble acetone extract C (11.8 g), light petroleum- and potas-

sium hydroxide-soluble acetone extract D (126 g), neutral light petroleum-soluble acetone extract E (516 g).

Sodium bicarbonate-soluble fraction. The oil C was mixed with half its volume of light petroleum and after several months deposited crystals (1.3 g) which were chromatographed on silicagel (130 g) impregnated with dimethyl sulphoxide¹⁰. Isopropyl ether eluted *hydroxy acid I* (180 mg), recrystallised from ether-light petroleum and sublimed in a high vacuum, m.p. 166–168°, $[\alpha]_D + 26^\circ$ (c, 2.2, ethanol (95 %)). (Found: C 72.2; H 9.1. $C_{15}H_{22}O_3$ requires C 72.0; H 8.9.) λ_{max} 298 μ , log ϵ 4.1. Acetone eluted *hydroxy acid II* (820 mg), recrystallised from ether-light petroleum and sublimed, m.p. 152–153°, $[\alpha]_D + 14^\circ$ (c, 1.7, ethanol (95 %)). (Found: C 72.3; H 8.1; O 19.5. $C_{15}H_{20}O_3$ requires C 72.6; H 8.1; O 19.3.) λ_{max} 237 μ , log ϵ 4.2; shoulder 269 μ ; λ_{max} 279 μ , log ϵ 2.8.

Selenium dehydrogenation. The methyl ester of hydroxy acid II (m.p. 67–68°) was prepared with diazomethane and reduced with lithium aluminium hydride to a diol (m.p. 156–157°). This was dehydrogenated with twice the amount of selenium at 280° for 36 h and the product was chromatographed on basic alumina. Light petroleum eluted only traces of a compound which gave an orange-red picrate. Ether eluted the main reaction product, λ_{max} 263 μ , E 500; shoulder 280 μ . Infrared spectrum (in KBr): 1 713 cm^{-1} s.

Alkali soluble fraction. The oil D was dissolved in ether and separated into a sodium carbonate-soluble oil F (85 g) and sodium carbonate-insoluble material G (40 g). The oil F gave no crystalline material on standing for a year. The oil G was dissolved in ligroin (100–125°) and shaken with a saturated solution of cupric acetate. The very small amount of copper salts formed was separated by filtration, washed with ligroin and water and decomposed with sulphuric acid (2 N). The acidic solution was extracted with ether and the ether solution was washed with water, dried and evaporated to give a reddish-brown oil (H, 16 mg). The ligroin filtrate was separated, washed with water, dried and evaporated to an oil (K).

The oils F, H and K were analysed by paper chromatography¹¹ but neither tropolones nor carvacrol could be detected in any of them.

Neutral fraction. By a fast preliminary distillation the neutral oil E was divided into a fraction L (297 g), b.p. up to 135°/1 mm, and a residue M. The oil L was redistilled through a 1 m, vacuum jacketed packed column giving the fractions listed in Table 1.

Table 1. Distillation of neutral fraction L. Total distillate 267 g or 90 %.

Fraction	Weight (g)	B.p./15 mm Hg (°C)	Rotation $[\alpha]_D$	Refractive index n_D^{25}
Ia	4.7	115–124	–29	1.4949
Ib	23.4	124–126	–64	1.4968
Ic	23.5	126	–78	1.4970
Id	18.7	126–127	–74	1.4970
Ie	23.3	127–139	–46	1.5019
If	19.2	139–142	–29	1.5094
Ig	24.6	142	–56	1.5089
Ih	24.6	142	–62	1.5061
Ik	23.5	142–145	–51	1.5047
Il	15.1	145–153	–42	1.5071
Im	13.7	153–155	0	1.5120
In	17.9	155–160	+11	1.5125
Io	19.1	160–166	–12	1.5155
Ip	15.9	166	–23	1.5155

Gas chromatographic examination of the sesquiterpene hydrocarbon fractions. Gas chromatograms were run on a Pye Argon Chromatograph as described in a previous paper¹².

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

Table 2. Gas chromatographic analysis of the sesquiterpene hydrocarbon fractions in Table 1. Argon flow rate 30 ml/min.

Fraction	Peak retention time (min)													
	4.3	7.7	10.7	11.6	14.1	15.9	20.4	23.8	27.3	33.9	36.4	41.2	45.4	52.7
Ia	1	9	31	4	52	3								
Ib		1	14		74	11								
Ic			3		81	16								
Id					78	22								
Ie					75	25								
If							10	54	2	1	8			25
Ig							4	30		2	10	2	2	50
Ih								20			9	2	2	67
Ik								4			4	4	3	85
Il											4	3	4	89

With the same flow rate the retention times of the following known compounds were: *a*-cedrene 14.1, δ -cadinene-*isocadinene* mixture⁶ (see below) 23.8, calamenene⁷ 36.4, sesquiterpene oxide 52.7.

Identification of a-cedrene. Fraction Id (3.0 g) was oxidised with selenium dioxide (1.8 g) as described in a previous paper¹⁴. The crude aldehyde (2.52) was further oxidised with silver oxide (3.91 g silver nitrate, 1.84 g sodium hydroxide) to give acidic material (1.72 g). This was chromatographed on silica gel (50 g) impregnated with dimethyl sulphoxide¹⁰. Isopropyl ether-light petroleum (1:2) eluted *a*-cedrenic acid⁴ (1.37 g) which was repeatedly sublimed in a high vacuum, m.p. 123.0–124.5° (identification by mixed m.p. and IR). The column was stripped with methanol but no other identifiable compound was obtained.

Maleic anhydride adduct. Fraction Ih (50 mg) in absolute benzene (5 ml) was treated with maleic anhydride (24 mg) giving an intensely yellow solution. This was refluxed for 3 h, the product was then precipitated by addition of light petroleum and recrystallised from benzene, m.p. 147–148° (13 mg).

Chromatography of the cadinene fraction. Fraction Ih (6.0 g) was chromatographed on basic alumina (600 g) which had been dried overnight at 150°. The first 460 ml of light petroleum eluted a δ -cadinene-*isocadinene*⁶ mixture (910 mg) which was distilled, n_D^{25} 1.5163, $[\alpha]_D -80^\circ$ (c, 2.2), λ_{\max} 247 m μ , ϵ 3.9. On dehydrogenation with an equal amount of selenium in an atmosphere of nitrogen at 270° for 24 h, a 60 % yield of cadalene (isolated as the picrate) was obtained.

The next 280 ml of light petroleum eluted calamenene⁷ (503 mg), which was distilled, n_D^{25} 1.5119, $[\alpha]_D -46^\circ$ (c, 1.7); λ_{\max} 265 m μ , $\log \epsilon$ 2.70; λ_{\max} 269 m μ , $\log \epsilon$ 2.75; λ_{\max} 273 m μ , $\log \epsilon$ 2.70; λ_{\max} 278 m μ , $\log \epsilon$ 2.72. Selenium dehydrogenation of this compound under the same conditions as above afforded a 76 % yield of cadalene.

Ether-light petroleum (1:1) eluted an *oxide* (3.73 g) which was distilled. The main fraction, b.p. 131°/12 mm, n_D^{25} 1.4967, $[\alpha]_D -69^\circ$ (c, 1.5) did not absorb above 220 m μ . (Found: C 81.6; H 10.9; CH₃O— 0.00; CH₃—(C) 5.6. C₁₅H₂₄O requires C 81.8; H 11.0; CH₃—(C) (one) 6.8.) On attempted selenium dehydrogenation 85 % of the starting material was recovered unchanged.

Sesquiterpene alcohols. Fraction Im (2.0 g) was chromatographed on basic alumina (150 g). Benzene (750 ml) eluted an oil (150 mg) which was discarded. Ether-benzene (1:20, 750 ml) eluted *cedrol* (700 mg), recrystallised from ethanol (95 %), m.p. 85–86°. Ether-benzene (1:1) eluted *widdrol*⁸ (340 mg) recrystallised from acetonitrile and sublimed in a high vacuum, m.p. 95–96° (identified by mixed m.p. and IR). Further elution with ether and with methanol gave oily fractions which have not been investigated.

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