

## The Chemistry of the Natural Order Cupressales XXVIII \* Constituents of *Juniperus virginiana* L.

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Commercial cedarwood oil contains cuparene, cedrol, widdrol,  $\alpha$ -cedrene and thujopsene. Evidence was obtained for the presence of a dextrorotatory curcumene containing a vinylidene group and gas chromatography indicated the presence of several other unidentified sesquiterpenes.

Investigations on the heartwood constituents of *Juniperus chinensis* L.<sup>1</sup> and *J. utahensis* Lemm.<sup>2</sup> have been described in two previous papers. Among the compounds identified in these juniper woods were the sesquiterpene hydrocarbons cuparene<sup>3</sup> and thujopsene<sup>4</sup> and the sesquiterpene alcohol widdrol<sup>5</sup>. The structures of the two latter compounds are at present under investigation in this laboratory. Experience gained in the examination of these juniper woods has now been applied to cedarwood oil<sup>6,7</sup>. Commercially this is the most important of the juniper wood oils.

Cedarwood oil is produced by steam distillation of saw mill waste of the American red cedar, *Juniperus virginiana*. The highest yield of oil, about 3.5 % of the wood, is obtained from older trees containing a greater proportion of heartwood. The crude oil is discoloured and the commercial product is obtained by redistillation of the crude material. The boiling point range of cedarwood oil has been given as 264—280° at atmospheric pressure. Several hundred thousand pounds of the oil are produced a year in the United States for use in perfumery and in scenting soaps.

Among the compounds previously reported in cedarwood oil is  $\alpha$ -cedrene but although this has been shown to be present it has probably never been isolated in a pure state from the natural oil. Natural cedrene has a lower negative specific rotation (usually about  $-55^\circ$ ) than the synthetic product ( $-91^\circ$ ) obtained by dehydration of cedrol<sup>8</sup>. The natural material is considered to be a mixture of  $\alpha$ -cedrene and the corresponding exocyclic isomer  $\beta$ -cedrene, but it has not been proved that the exocyclic compound present

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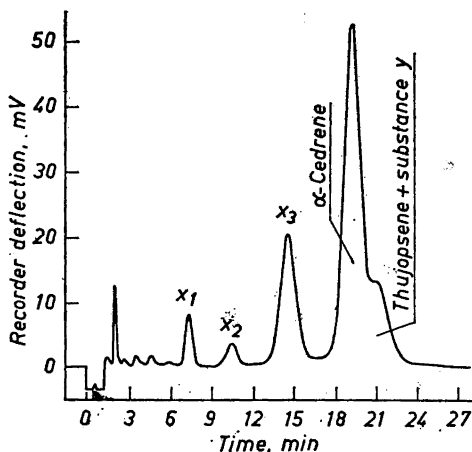


Fig. 1. Gas chromatogram of fraction IIa (Table 2). Argon flow rate 25 ml/min.

actually has the cedrene skeleton. Cedrol has frequently been isolated from *J. virginiana* and cedrenol<sup>9</sup> (of. Ref.<sup>10</sup>) and pseudocedrol<sup>11</sup> have also been reported to occur in the wood.

The commercial sample of cedarwood oil used in this investigation was subjected to a preliminary distillation followed by redistillation of some of the fractions obtained. Traces of monoterpenes or compounds with similar boiling points were detected in the lowest boiling fractions by gas chromatography (see Fig. 1, peaks with retention times up to about 11 min.). Only minute amounts of compounds containing hydroxyl groups were present in these fractions. Infrared spectra and gas chromatographic analysis showed that  $\alpha$ -cedrene was the main component of the oil. The second largest constituent was thujopsene which has not previously been demonstrated in cedarwood oil. One of the main fractions was oxidised with selenium dioxide to a mixture of aldehydes, and then with silver oxide to yield the corresponding carboxylic acids. Chromatography of this mixture gave  $\alpha$ -cedrenic acid<sup>12</sup> and hinokiic acid (thujopsenic acid<sup>4</sup>).

Thujopsene was almost completely separated from  $\alpha$ -cedrene by fractional distillation but gas chromatographic analysis showed that both fractions still contained other compounds.

The specific rotations of the distillation fractions listed in Table 2 indicate that in addition to  $\alpha$ -cedrene ( $-91^\circ$ ) and thujopsene ( $-112^\circ$ ) there are at least two further compounds present, probably with positive rotations. One is concentrated in the first fractions of Table 2 and gives a separate peak on gas chromatography, the other is concentrated in the last fractions and is apparently covered by the thujopsene peak.

Two of the fractions of Table 2 were dehydrogenated and then treated with ozone at low temperature. The material unattacked by ozone was optically inactive and it was identical with a synthetic sample of dihydro-*ar*-curcumene<sup>13</sup>. Neither  $\alpha$ -cedrene nor thujopsene gave dihydro-*ar*-curcumene when treated in the same way. *d*- $\gamma$ -Curcumene<sup>14</sup>, which gives dihydro-*ar*-

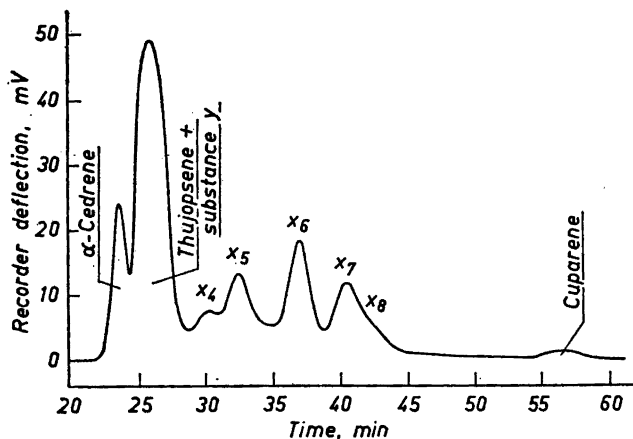


Fig. 2. Gas chromatogram of fraction IIIc (Table 3). Argon flow rate 17 ml/min.

curcumene on selenium dehydrogenation<sup>15</sup> was at first considered a possible precursor of this substance. Since, however, the ultraviolet spectra of the two fractions, before dehydrogenation, show maxima only at 205 and 207  $m\mu$ , respectively, they cannot contain any major amounts of *d*- $\gamma$ -curcumene. However, the infrared spectra of both fractions show comparatively strong bands at 1645 and 883  $cm^{-1}$  indicating the presence of a compound containing a vinylidene group. It therefore appears likely that the dihydro-*ar*-curcumene precursor (substance *y*, Figs. 1 and 2) is an isomer of curcumene containing a vinylidene group.

An attempt was made to purify the above sesquiterpenes by chromatography on basic alumina. The first fractions eluted by light petroleum were reasonably pure  $\alpha$ -cedrene but thujopsene could not be separated from the other components of the mixtures.

Naves has previously reported the presence of dextrorotatory bicyclic sesquiterpenes in *J. virginiana*<sup>16</sup>, and one of these has now been identified. The material boiling slightly higher than the main  $\alpha$ -cedrene-thujopsene fraction was redistilled and a fraction showing strong aromatic absorption in the infrared and ultraviolet was ozonised at low temperature. The material unattacked by ozone was identified as cuparene<sup>3</sup>.

Gas chromatographic analysis of the cuparene containing fractions showed the presence of at least 7 unidentified compounds. The separation of these compounds on a larger scale offers considerable difficulties but could probably be achieved by preparative gas chromatography. No isomerisation of these compounds on the stationary phase used was observed.

The highest boiling part of cedarwood oil was a mixture of sesquiterpene alcohols. The first fractions containing alcohols (fraction IIIh (Table 3) and fractions IVa—IVc (Table 4)) crystallised on standing and were separated into cedrol and widdrol by chromatography on alumina. The highest boiling

alcohol fractions remained liquid and appeared, according to their infrared spectra to consist of a complex mixture of alcohols and carbonyl compounds.

The compounds isolated are listed below with very approximate estimates of the amounts present (as a percentage of dried cedarwood oil):  $\alpha$ -Cedrene 35, thujopsene 30, cuparene 2, cedrol 4 and widdrol 2.

### EXPERIMENTAL

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

The cedarwood oil (Ia, Florida, redistilled) used was obtained from the Dragoco Co, Holzminden, Weser, Germany. The oil was dried over sodium sulphate and a sample (2010 g) was then subjected to a preliminary distillation through a 40 cm, packed column. The fractions obtained are listed in Table 1.

Table 1. Distillation of cedarwood oil. Total distillate 1 729 g or 86 %

Fraction	Weight (g)	B.p. (°C)	Pressure (mm Hg)	Rotation ([ $\alpha$ ] <sub>D</sub> )	Refractive index ( $n_D^{25.0}$ )
Ia	181	74–114	11	–57	1.4999
Ib	250	114–118	11	–60	1.5018
Ic	399	82–83	1	–66	1.5007
Id	343	83–84	1	–58	1.5011
Ie	325	84–89	1	–47	1.5024
If	231	89–111	1	+ 4	1.5077
Ig (residue)	271				

Fractions Ia (65 g) and Ib (237 g) were combined and distilled through a 1 m, vacuum jacketed, packed column giving the fractions listed in Table 2.

Table 2. Redistillation of the combined fractions Ia and Ib. Total distillate 283 g or 94 %

Fraction	Weight (g)	B.p./14 mm Hg (°C)	Rotation ([ $\alpha$ ] <sub>D</sub> )	Refractive index ( $n_D^{25.0}$ )
IIa	19.3	76–124	–30	1.4966
IIb	37.8	124	–55	1.4981
IIc	28.8	124–125	–66	1.4983
IId	41.0	125	–74	1.4990
IIe	55.8	125	–80	1.4996
IIf	23.0	125	–81	1.5001
IIg	46.6	125–126	–85	1.5008
IIh	30.4	126–129	–39	1.5053

*Dehydrogenations.* (a) Dihydro-*ar*-curcumene was obtained from fraction IIb (5 g) by heating with finely powdered selenium (5.0 g) to 200° in a carbon dioxide atmosphere; the temperature was gradually raised to 280° and held there for 48 h. The reaction product was filtered and distilled (b.p. 122–129°/10 mm) and the oil obtained (4.3 g) was ozonised in methylene chloride (20 ml) at –70°C until an excess of ozone was indicated by the appearance of a blue colour. When the reaction mixture had reached room temperature, zinc dust (4.7 g) activated with glacial acetic acid (1 ml) was added with stirring and the mixture was left over-night and filtered. Hydrogen peroxide (30 %, 10 ml) in potassium hydroxide (10 %, 5 ml) was added dropwise to the filtrate and the mixture was refluxed for 5 min. The aqueous phase was separated and the organic phase was extracted with sodium hydroxide (2 N), washed with water, dried and evaporated giving

a viscous oil (3.4 g). This was chromatographed on basic alumina (40 g). The first 100 ml of light petroleum eluted a hydrocarbon fraction which on distillation under reduced pressure gave an oil (0.95 g), b.p. 126°/9 mm,  $[\alpha]_D 0^\circ$ ,  $d_{22.0} 0.8767$ ,  $n_D^{25.0} 1.4862$ , which was identified as dihydro-*ar*-curcumene<sup>15,17</sup>. The infrared spectra of the dehydrogenation product and of a synthetic sample<sup>18</sup> were superimposable. On treatment with aqueous nitric acid (30 %) in a sealed tube at 170° followed by methylation with diazomethane in ether, the dehydrogenation product gave dimethyl terephthalate, m.p. and mixed m.p. 140.0–140.5°.

(b) Fraction IIIh (5.0 g) was dehydrogenated and ozonised in the same way as IIb. The material unattacked by ozone (1.57 g) was identified as dihydro-*ar*-curcumene.

*a*-Cedrene and thujopsene. Fraction Id (30 g) was dissolved in ethanol (95 %, 500 ml), selenium dioxide (18.0 g) was added and the mixture was refluxed for 5 h. After filtering, the solution was evaporated and the oil obtained was distilled, b.p. 95–115°/1.0 mm (21.8 g). This oil (17.5 g) was added to a suspension of silver oxide (sodium hydroxide 12.9 g, silver nitrate 28.8 g) in water (600 ml). The mixture was refluxed with vigorous stirring for 24 h. Solid material was filtered off and the filtrate was extracted with ether, acidified and again extracted with ether. This second extract, on evaporation gave a crystalline residue (A, 6.4 g).

Silica gel (30 g) suspended in a solution of dimethyl sulphoxide in chloroform (5 %, 100 ml) was poured into a chromatographic column and then washed successively with isopropyl ether and light petroleum. The crude acids A (916 mg) were chromatographed on this pretreated silica gel using an isopropyl ether-light petroleum mixture (1:2) as mobile phase. The first 250 ml of solvent eluted a crystalline substance (149 mg) which was sublimed three times in a vacuum giving *a*-cedrenic acid<sup>12</sup>, m.p. 123.5–125.0°,  $[\alpha]_D -69^\circ$  (c, 2.0) (identified by mixed m.p. and IR). The next 150 ml of solvent eluted a second compound (240 mg) which was recrystallised from light petroleum, m.p. 166.5–167.5°,  $[\alpha]_D -85^\circ$  (c, 2.4), identified as hinokiic acid<sup>4</sup> (mixed m.p., IR). The rest of the chromatographed sample appeared to be polymeric material.

*Cuparene*. Cuparene was concentrated by redistillation of the highest boiling fraction in Table 1 (I f, 210 g).

Table 3. Redistillation of fraction If. Total distillate 192 g or 91 %.

Fraction	Weight (g)	B.p./14 mm Hg (°C)	Rotation ( $[\alpha]_D$ )	Refractive index ( $n_D^{25.0}$ )
IIIa	18.4	124–126	–77	1.5000
IIIb	28.7	126–127	–84	1.5012
IIIc	22.0	127–134	–42	1.5050
IIId	27.3	134–136	+32	1.5102
IIIe	24.6	136–138	+43	1.5116
IIIf	25.1	138–139	+42	1.5109
IIIg	11.4	139–147	+42	1.5091
IIIh	34.8	147–153	+31	–

Fraction IIIf (5.0 g) was ozonised and treated in the same way as the dehydrogenation product of fraction IIb. The material unaffected by ozone (810 mg), b.p. 117°/7 mm,  $[\alpha]_D +65^\circ$ ,  $n_D^{25.0} 1.5206$ , was identified as cuparene<sup>3</sup> by comparing its physical constants and infrared spectrum with those of an authentic sample.

*Cedrol and widdrol*. A sample of the crystalline fraction IIIh (2.5 g) was chromatographed on basic alumina (40 g). Light petroleum eluted an oil (0.17 g) which was not further investigated, 2 % ether in benzene gave cedrol (1.45 g) which was recrystallised from 95 % ethanol, m.p. 85.0–85.5°,  $[\alpha]_D +11^\circ$  (identified by mixed m.p. and IR). Elution with benzene-ether (1:1) gave widdrol<sup>5</sup> (0.56 g) which was recrystallised from acetonitrile, m.p. 95.0–96.0°,  $[\alpha]_D +105^\circ$  (identified by mixed m.p. and IR).

*Main sesquiterpene alcohol fraction*. The residue (I g, 271 g) from the preliminary distillation described in Table 1 was distilled in a 1 m, vacuum-jacketed, packed column, giving the fractions listed in Table 4.

Table 4. Distillation of the main sesquiterpene alcohol fraction. Total distillate 214 g or 79 %.

Fraction	Weight (g)	B.p./14 mm Hg (°C)	Rotation ([ $\alpha$ ] <sub>D</sub> )
IVa	19.2	134–154	+30
IVb	26.9	154	+31
IVc	88.6	154–156	+26
IVd	39.5	156–163	+ 4
IVe	40.0	163	+24

Gas chromatographic examination of the sesquiterpene hydrocarbon fractions. A Pye Argon Chromatograph, Cat. No. 12000, was used together with a Philips Automatic Compensator PR 2210 A/21 (Column length 1.20 m, internal column diameter 5 mm, stationary phase 100–115 mesh Silocel C 22 brick powder impregnated with 2,4-dinitrophenyl-2-naphthyl ether (15 %) and dibenzylpyridine (a mixture of 2,4- and 2,6-isomers, 0.75 %) <sup>19</sup>, temperature 150°, charge 0.025  $\mu$ l).

With an argon flow rate of 25 ml/min the retention times (min) were:  $\alpha$ -cedrene 19.1 (reference sample prepared from cedrol), thujopsene 21.0, dihydro-*ar*-curcumene 23.8, cuparene 46.0. In the Tables 5–7 the approximate areas of individual peaks of different retention times are given as percentages of total peak area.

Table 5. Gas chromatographic analysis of the fractions in Table 1. Argon flow rate 25 ml/min.

Fraction	Peak retention time (min)							
	14.6	19.1	21.0	27.0	30.4	33.2	37.6	46.0
Ia	2	55	43					
Ib	2	53	45					
Ic	1	51	48					
Id		49	51					
Ie		44	54			2		
If		18	32	2	7	27	2	12

Table 6. Gas chromatographic analysis of the fractions in Table 2. Argon flow rate 25 ml/min.

Fraction	Peak retention time (min)					
	14.6	19.1	21.0	27.0	30.4	33.2
IIa*	20	56	16			
IIb	9	69	22			
IIc	3	66	31			
IId		65	35			
IIe		59	41			
IIf		48	52			
IIg		29	71			
IIh		11	67	3	8	11

\* 8 % of total peak area of this fraction corresponds to several small peaks with retention times similar to those of monoterpenes (cf. Fig. 1).

Table 7. Gas chromatographic analysis of the fractions in Table 3.  
Argon flow rate 17 ml/min.

Fraction	Peak retention time (min)										
	18.4	23.8	26.0	30.1	32.5	36.9	40.3	42.1	46.9	54.9	56.4
IIIa	1	46	50	3							
IIIb		38	57	4		1					
IIIc*		11	41	8	12	13	10	4			1
IIId			1	3	7	21	34	22	4		8
IIIe					1	9	35	27	7	5	16
IIIf						2	23	21	7	20	27
IIIg						1	4	8	8	21	58

\* Cf. Fig. 2.

With an argon flow rate of 17 ml/min the retention times (min) were: *a*-cedrene 23.8, thujopsene 26.0 and cuparene 56.4.

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