ACTA CHEMICA SCANDINAVICA 15 (1961) 713–720

The Chemistry of the Natural Order Cupressales

XXXIV.* Heartwood Constituents of *Juniperus procera* Hochst. and *Juniperus californica* Carr.

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The heartwood of *Juniperus procera* contains a new sesquiterpene hydroxy acid, a diterpene phenol, the tropolone procerin, three other tropolones, carvacrol, α-cedrene, cuparene, cedrol, β-sitosterol and a C_{30}-compound previously isolated from *J. thurifera*.

The heartwood of *Juniperus californica* contains hinokiic acid, "Widdringtonia acid II", α-cedrene, thujopsene, cuparene, cedrol and widdrol.

Gas chromatography indicated the presence of several unidentified sesquiterpenes in both species.

With the exception of *J. procera* Hochst., a native of Kenya, all the species of the genus *Juniperus* are found in the northern hemisphere. *J. procera* is widespread in the equatorial highlands of Africa and in Abyssinia, thus occurring on both sides of the equator.

In Kenya it grows in the drier forests at altitudes of 5 000 to 6 800 ft. It is a big tree attaining 80—100 ft in height and sometimes 35 ft in girth.[1]

In the present series of papers [2] the heartwoods of a number of northern hemispheric junipers have been investigated. It was therefore of interest to investigate the pattern of heartwood constituents of *J. procera* which has probably spread from the north across the equator.

Cedrol, cedrene and *l*-limonene have previously been reported to occur in this species [3].

J. californica Carr. is a much branched shrub or small tree growing chiefly on the coastal mountains of Central and Southern California [4].

Both species were worked up essentially in the same way. The wood was extracted with acetone and the light petroleum-soluble part of the acetone extract was separated into sodium bicarbonate-, sodium carbonate-, potassium hydroxide-soluble and neutral fractions.

* Part XXXIII. *Acta Chem. Scand.* 15 (1961) 645.

A

B

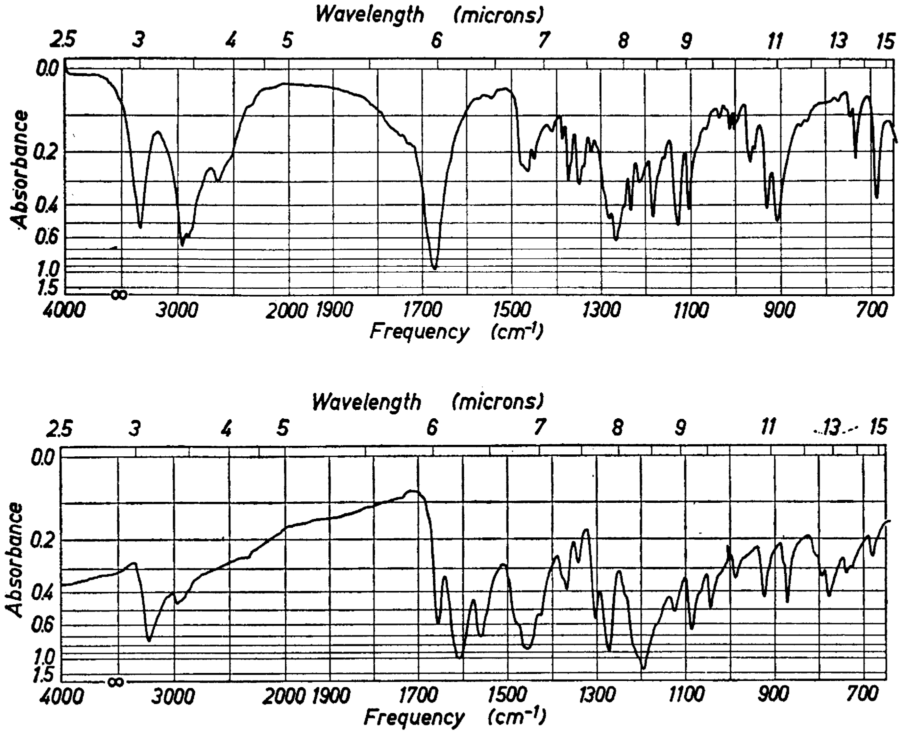

Fig. 1. Infrared spectra of hydroxy acid, m.p. 260—261° (A) and phenol, m.p. 149—151° (B) in potassium bromide.

J. procera. The wood used in this investigation was obtained from Kenya.

The sodium bicarbonate-soluble material afforded a new sesquiterpene hydroxy acid, m.p. 260—261°, and a diterpene phenol, m.p. 149—151°. The infrared spectra of these compounds are shown in Fig. 1.

The sodium carbonate-soluble fractions of several junipers contain two sesquiterpene acids, hinokiic acid [5] and "Widdringtonia acid II" [6,2] (*cf.* below). The structure of the latter compound is still unknown. In the corresponding fraction from *J. procera* neither of these acids could be detected, but the fraction afforded a new C_{15}-tropolone which was named procerin. The elucidation of its structure has been described in a previous paper [7]. Chromatography of the rest of the sodium carbonate-soluble fraction gave three other compounds. These were isolated in such small amounts that analyses could not be obtained but spectroscopic evidence and paper chromatographic behaviour indicated that they were all new tropolones. According to infrared data two of the compounds contain an unsymmetrically substituted vinylidene group.

None of the tropolones common to other junipers investigated could be detected in *J. procera* by paper chromatography.

The tropolone fraction of *J. procera* thus differs remarkably from those of all other junipers investigated. The fungicidal properties of tropolones are well established [8] and most tropolone-producing conifers are known for the resistance of their heartwood to micro-organisms. The wood of *J. procera* is reported to be especially durable and to resist attack by insects [1]. This species, being adapted to the equatorial region, might well have developed a set of tropolones particularly effective against the attacks of micro-organisms and insects occurring in a tropical climate.

Further work on the tropolones of this species is in progress.

The neutral oil was distilled. Only traces of monoterpenes or compounds with similar boiling points could be detected in the lowest boiling material. The main constituent of the sesquiterpene hydrocarbon fraction was α-cedrene which was identified by conversion to α-cedrenic acid [9]. No thujopsene [5] could be detected. This was unexpected since all junipers examined in the present series of investigations [2] contained this compound. It is possible, however, that very small amounts of thujopsene occur in *J. procera.*

According to gas chromatography and infrared data the second largest component of the sesquiterpene hydrocarbon fraction could be identical with hydrocarbon X_2 from *J. thurifera* [10].

From the next higher boiling fraction a small amount of cuparene [11] was isolated.

The large crystalline sesquiterpene alcohol fraction that followed apparently consisted of cedrol only. The highest boiling neutral material remained liquid.

The neutral distillation residue yielded two crystalline compounds which were separated by sublimation.

These were β-sitosterol and a C_{30}-compound, the "triterpene diol", m.p. 243—244°, previously isolated from a similar fraction of *J. thurifera* [10].

According to its infrared spectrum, the remainder of the neutral distillation residue appeared to contain a complex mixture of alcohols and carbonyl compounds.

The compounds isolated are listed below with very approximate estimates of the amounts present (as percentages of the air-dried wood). Total acetone extract 9.4, ether-soluble acetone extract 2.9, light petroleum-soluble acetone extract 2.1, sodium bicarbonate-soluble 0.04, potassium hydroxide-soluble 0.5, neutral 1.6, sesquiterpene hydroxy acid 0.0004, diterpene phenol 0.0008, procerin 0.1, tropolone II 0.02, tropolone III 0.004, tropolone IV 0.0007, carvacrol 0.04, α-cedrene 0.5, cuparene 0.03, cedrol 0.5, β-sitosterol 0.001, "triterpene diol" 0.0007.

J. californica. The wood used in this investigation was collected in the Mohave Desert, California, USA.

The sodium carbonate-soluble fraction gave hinokiic acid [5] and "Widdringtonia acid II" [6]. Rather surprisingly, considering the close relationship of *J. californica* and *J. utahensis* [12,13], no known tropolones could be detected in the former species.

The neutral oil was distilled and the fractions analysed by infrared spectroscopy and gas chromatography. Thujopsene [5] was the major component of the sesquiterpene hydrocarbon fraction which also contained α-cedrene and cuparene [11] in addition to several unidentified compounds.

The crystalline sesquiterpene alcohol fraction that followed was separated into cedrol and widdrol [6] by chromatography.

The highest boiling fraction remained liquid, and from the distillation residue a small amount of a compound, m.p. 241—242°, was isolated. According to the infrared spectrum its carbon skeleton appeared to be very similar to that of the "triterpene diol", m.p. 243—244°, of *J. procera* and *J. thurifera* [10] (*cf.* above), but the hydroxyl absorbtion differed. The melting point of the former compound was depressed on admixture of the "triterpene diol".

The compounds isolated are listed below with very approximate estimates of the amounts present (as percentages of the air-dried wood). Total acetone extract 4.0, ether-soluble acetone extract 2.2, light petroleum-soluble acetone extract 1.3, sodium bicarbonate-soluble 0.05, potassium hydroxide-soluble 0.4, neutral 0.9, hinokiic acid 0.03, "Widdringtonia acid II" 0.02, α-cedrene 0.01, thujopsene 0.1, cuparene 0.004, cedrol 0.2 and widdrol 0.0006.

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 m.p. 40—60°.

Juniperus procera

The air-dried heartwood (24.4 kg) was extracted with acetone for 24 h and fractionated as described in a previous paper in this series [14]. Ether-insoluble acetone extract A (1 480 g), ether-soluble but light petroleum-insoluble acetone extract B (194 g), light petroleum- and sodium bicarbonate-soluble acetone extract C (9.8 g), light petroleum- and potassium hydroxide-soluble acetone extract D (117 g), light petroleum-soluble neutral acetone extract E (395 g).

Sodium bicarbonate-soluble fraction. The oil C was mixed with half its volume of light petroleum and on standing for several months deposited a small amount (324 mg) of crystalline material, which on fractional crystallisation from acetonitrile afforded a less soluble (101 mg) and a more soluble (210 mg) product. The less soluble material was recrystallised from acetonitrile and sublimed in a high vacuum to give a *hydroxy acid* (45 mg), m.p. 260—261°, $[\alpha]_D -30°$ (*c*, 1.9, pyridine). (Found: C 71.4; H 9.4; O 19.3; active H (Zerevitinoff) 0.81; $CH_3-(C)$ 4.6. $C_{15}H_{24}O_3$ requires C 71.4; H 9.6; O 19.0; active H (two) 0.80; $CH_3-(C)$ (one) 6.0.) The hydroxy acid did not exhibit any significant absorbtion in the 220—350 mμ range.

The more soluble material was recrystallised from ethanol (95 %) and sublimed in a high vacuum, giving pale greenish crystals of a *phenol* (120 mg), m.p. 149—151°, $[\alpha]_D +7°$ (*c*, 1.7). (Found: C 73.8; H 6.7; O 19.7; CH_3O—0.00; active H 0.69. $C_{20}H_{22}O_4$ requires C 73.6; H 6.8; O 19.6; active H (two) 0.62.) λ_{max} 230 mμ, log ε 4.37; λ_{max} 245 mμ, log ε 4.35; shoulder 265 mμ; λ_{max} 364 mμ, log ε 4.24; λ_{max} 398 mμ, log ε 4.26. The compound in methanol gave a greenish colour with methanolic ferric chloride.

Alkali-soluble fraction. The oil D was dissolved in ether and separated into a sodium carbonate-soluble fraction F (98.0 g) and a sodium carbonate-insoluble oil G (18.6 g). Fraction F partly crystallised on standing. This material was recrystallised from light petroleum and sublimed to give tropolone I (procerin [7], 8.6 g). On standing for several months fraction F yielded an additional amount (10.7 g) of crude procerin. The mother liquors from recrystallisation of procerin were evaporated and combined with the non-crystalline part of fraction F. A sample (1.85 g) of the combined material (H) was chromatographed on silica gel (120 g) impregnated with dimethyl sulphoxide (*cf.* Ref. [15]) using a mixture of light petroleum-dimethyl sulphoxide as eluent (light petroleum (1 part), light petroleum saturated with a solution of water (4 %) in dimethyl sulphoxide (4 parts)). The first 1 600 ml of solvent eluted procerin (410 mg), the next 500 ml eluted a crystalline

Table 1. Distillation of neutral fraction K. Total distillate 284 g or 86 %.

Fraction	Weight (g)	B.p./15 mm (°C)	Rotation ($[\alpha]_D$)	Refractive index (n_D^{25})
Ia	13.4	117—127	—51	1.4972
Ib	27.8	127—128	—75	1.4966
Ic	35.1	128	—74	1.4965
Id	27.4	128	—76	1.4967
Ie	21.1	128—135	—59	1.4981
If	22.2	135—158	5	—
Ig	53.8	158	12	—
Ih	57.4	158—159	8	—
Ii	25.9	159—166	—16	1.5186

substance (70 mg) which was repeatedly sublimed in a high vacuum to give tropolone II, (5 mg) m.p. 133—134°. λ_{max} 242 mμ, *E* 1 050 (for nomenclature *cf.* Ref. [16]); shoulder 290 mμ; shoulder 324 mμ; λ_{max} 334 mμ, *E* 305; λ_{max} 351 mμ, *E* 270; λ_{max} 367 mμ, *E* 370. Infrared spectrum (in KBr): 3 205 s, 1 645 m, 1 610 s, 1 554 s, 1 282 s, 1 217 s, 907 w, 888 cm^{-1} m. The next 500 ml of solvent eluted a bright yellow substance containing *tropolone III* (20 mg) which could not be obtained in a pure state, m.p. 119—127°. λ_{max} 228 mμ, *E* 880; λ_{max} 300 mμ, *E* 1 050; λ_{max} 313 mμ, *E* 1280; λ_{max} 327 mμ, *E* 790; λ_{max} 357 mμ, *E* 270; λ_{max} 370 mμ, *E* 340; λ_{max} 385 mμ, *E* 340; λ_{max} 410 mμ, *E* 220. Acetone-light petroleum (1:9) eluted *tropolone IV* (3 mg) which was sublimed in a high vacuum, m.p. 72—74°. λ_{max} 243; shoulder 334 mμ; λ_{max} 347 mμ; λ_{max} 369 mμ; shoulder 393 mμ. On paper chromatography [17] the following R_F values were obtained: procerin 0.72 (dark brown colour reaction with *bis*-diazotised benzidine), nootkatin 0.72, tropolone II 0.50 (brown), tropolone III 0.52 (brown, main spot) and 0.43 (violet, minor spot), tropolone IV 0.43 (light brown, weak colour), β-thujaplicin 0.39, γ-thujaplicin 0.30. In addition to these spots, the crude material H gave a fairly large spot with R_F 0.34 (orange red) and several minor spots with still lower R_F values.

The sodium carbonate-insoluble oil G had the characteristic smell of *carvacrol* and paper chromatography [17] indicated the presence of this compound (R_F 0.21).

Neutral fraction. By a fast preliminary distillation the neutral oil E was divided into fraction K, b.p. up to 140°/1 mm (330 g) and a residue L (61 g). The oil K was redistilled through a 1 m, vacuum-jacketed, packed column giving the fractions listed in Table 1.

Gas chromatographic analysis of the sesquiterpene hydrocarbon fractions. Gas chromatograms were run on a Pye Argon Chromatograph as described in a previous paper in this series [18] (temperature 150°, charge 0.025 μl).

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 20 ml/min.

Fraction	Retention time (min)								
	5.4	16.3	18.2	21.2	24.2	26.8	28.9	36.4	51.1
Ia	2	4	2	87	4			1	
Ib		2		91	7				
Ic				93	7				
Id				90	10				
Ie				78	16	3	3		
If (liquid part)									30

With the same argon flow rate the retention times of the following known compounds were: α-cedrene 21.2, thujopsene [5] 23.4 and cuparene [11] 51.1 min.

Identification of α-cedrene. Fraction Ie (6.0 g) was dissolved in ethanol (95 %, 80 ml), selenium dioxide (3.6 g) was added and the mixture was refluxed for 3 h. After filtering, the solution was evaporated and the oil obtained was distilled in a high vacuum giving a pale greenish distillate (4.6 g). This (2.0 g) was added to a solution of silver nitrate (3.1 g) in water-dioxane (1:6). A solution of sodium hydroxide (1.1 g) in water-dioxane (1:1) was added dropwise with stirring and the reaction mixture was then stirred for 1 h at room temperature followed by 21 h under reflux. After acidification (H_2SO_4, 2 N), water (500 ml) was added and the solution was extracted with ether. The ether solution was extracted with sodium carbonate (2 N), the alkaline solution was acidified and extracted with ether. The ether extract on evaporation gave a crystalline residue (1.5 g).

This residue (1.5 g) was chromatographed on silica gel impregnated with dimethyl sulphoxide [15]. *Iso*propyl ether-light petroleum (1:2) eluted a compound (1.1 g) which was repeatedly sublimed in a high vacuum, m.p. 124–125°, $[\alpha]_D$ −68° (*c*, 2.1), identified as α-cedrenic acid [9] (mixed m.p., I.R.). No other identifiable compound could be eluted and the rest of the chromatographed sample was a noncrystallisable gum.

Cuparene. The crystalline part of fraction If (Table 1) was filtered off and the filtrate (4.9 g) ozonised and treated as described in a previous paper [18]. The material unaffected by ozone (0.23 g), b.p. 119°/10 mm, $[\alpha]_D$ +59° (*c*, 2.5), n_D^{25} 1.5112 was identified as cuparene [10] by comparing its infrared spectrum and gas chromatographic data (see above) with those of an authentic sample.

Cedrol. The crystalline fraction Ih (Table 1, 2.0 g) was chromatographed on basic alumina (80 g). Ether-benzene (1:99) eluted a compound (1.9 g) which was recrystallised from ethanol (95 %), m.p. 85.5–86.0°, $[\alpha]_D$ +10° (*c*, 3.0) and identified as cedrol (mixed m.p., I.R.). The column was eluted with ether-benzene mixtures containing an increasing amount of the former solvent, and finally with methanol, but no further compounds were obtained.

β-Sitosterol. On standing for about 8 months the neutral distillation residue L deposited a small amount of crystals. These were washed with benzene and the remaining material (0.19 g) was separated into two compounds by repeated sublimation in a high vacuum along a temperature gradient. One of these (60 mg) was purified by further sublimation, m.p. 135–136°, and identified (mixed m.p., I.R.) as β-sitosterol.

C_{30}-compound. The other compound (40 mg) was recrystallised from benzene and sublimed in a high vacuum, m.p. 243–244°. It was identified (mixed m.p., I.R.) with "compound III" previously isolated from a similar fraction obtained from the heartwood of *Juniperus thurifera* [10].

Juniperus californica

The air-dried heartwood (4.78 kg) was extracted with acetone for 24 h and fractionated as previously described [14]. Ether-insoluble acetone extract A (88 g), ether-soluble but light petroleum-insoluble acetone extract B (42 g), light petroleum- and sodium bicarbonate-soluble acetone extract C (2.3 g), light petroleum- and potassium hydroxide-soluble acetone extract D (19.1 g), light petroleum-soluble neutral acetone extract E (41.1 g).

Alkali-soluble fraction. The potassium hydroxide-soluble oil D was dissolved in ether and extracted with sodium carbonate (2 N). The ether solution was washed with water, dried and evaporated to yield an oil F (4.6 g). On acidification and extraction with ether the sodium carbonate solution gave an oil G (14.0 g) which partly crystallised on standing. The crystals (1.27 g) were separated by filtration (mother liquor, H) and chromatographed on dried silica gel (15 g). The first 200 ml of light petroleum eluted oily material (28 mg), the next 200 ml eluted a compound (580 mg) which was repeatedly sublimed in a high vacuum, m.p. 164–166°, and identified (mixed m.p., I.R.) as *hinokiic acid* [5]. Ether-light petroleum (1:25) eluted another compound (450 mg) which was recrystallised from ethanol-water, sublimed in a high vacuum, m.p. 190–192°, and identified as the "*acid II*" isolated from *Widdringtonia* species [6]. When the column was stripped with methanol a small amount of an oil was obtained which was not further investigated.

Table 3. Distillation of neutral fraction K. Total distillate 20.2 g or 72 %.

Fraction	Weight (g)	B.p./15 mm (°C)	Rotation ($[\alpha]_D$)	Refractive index n_D^{25}
IIIa	1.59	115–126	–72	1.4988
IIIb	1.98	126–127	–90	1.5019
IIIc	1.83	127–128	–71	1.5047
IIId	3.28	128–147	7	1.5079
IIIe	2.02	147–159	10	—
IIIf	3.57	159	11	—
IIIg	2.35	159	15	—
IIIh	1.71	159–163	8	—
IIIi	1.85	163–166	7	1.5118

The oils F and H were analysed by paper chromatography [17] but no known tropolones were detected. The oil F gave a distinct spot with approximately the same R_F value as β-thujaplicin (0.39) but with a different colour reaction (bright red). Another major component of the oil gave a spot with an R_F value (0.24) slightly larger than that of carvacrol (0.21) and a similar colour reaction (orange red) but no carvacrol could be detected.

Neutral fraction. By a fast preliminary distillation the neutral oil E was divided into fraction K, b.p. up to 150°/1.3 mm (27.9 g) and a residue L (13.0 g). The oil K was redistilled through a 1 m spinning band column giving the fractions listed in Table 3.

Gas chromatographic analysis of the sesquiterpene hydrocarbon fractions. Gas chromatograms were run as described in a previous paper in this series [18] (temperature 150°, charge 0.025 μl).

In Table 4 the approximate areas of individual peaks of different retention times are given as percentages of total peak area.

With the same argon flow rate the retention times of the following known compounds were: *α-cedrene* 13.2, *thujopsene* [5] 14.3 and *cuparene* [11] 29.7 min. The presence of the above compounds in this species was confirmed by the infrared and ultraviolet spectra of the distillation fractions in Table 3.

Sesquiterpene alcohols. A sample of the crystalline fraction IIIg (1.0 g) was chromatographed on basic alumina (50 g). Ether-benzene (1:99) eluted a compound (970 mg) which was recrystallised from ethanol (95 %), sublimed in a high vacuum, m.p. 85–86°, and identified (mixed m.p. IR) as *cedrol.* Ether eluted another crystalline compound (3 mg) which was recrystallised from acetonitrile and sublimed, m.p. 92.5–94.5°, undepressed by an authentic sample of *widdrol* [6].

On standing for several months the neutral distillation residue L deposited a small amount of crystals (30 mg). This was recrystallised from benzene and sublimed in a high vacuum, m.p. 241–242°. Infrared spectrum (in KBr): 3 620 w, 3 500 m, 3 295 s, 1 275 m, 1 256 w, 1 236 w, 1 211 cm^{-1} w.

Table 4. Gas chromatographic analysis of the sesquiterpene hydrocarbon fractions in Table 3. Argon flow rate 34 ml/min.

Fraction	Retention time (min)									
	10.5	11.1	13.2	14.3	17.9	20.1	22.3	25.1	29.7	34.1
IIIa	1	4	13	82						
IIIb			9	91						
IIIc			4	83	2	2	6		3	
IIId			2	32	4	11	23	5	17	6

Acknowledgements. Our thanks are due to Professor H. Erdtman for his interest in this work, to the Tropical Products Institute, London, for generous gifts of wood of *J. procera*, to Docent O. Theander, Stockholm, for collecting the wood sample of *J. californica* used in this investigation, to Miss G. Hammarberg for infrared and ultraviolet spectra and to the *Swedish Natural Science Research Council* for financial support.

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Received December 2, 1960.