

The Chemistry of the Natural Order Cupressales

XXXVI. The Ethereal Oil of the Wood of *Juniperus communis* L.

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From the ethereal oil of the wood of *Juniperus communis* L. thujopsene, cuparene, humulene, cedrol, widdrol, and an α,β -unsaturated aldehyde have been isolated. Chromatographic and spectroscopic evidence has been obtained for the presence of calamenene, δ -cadinene, an isocadinene, α - and β -thujaplicin and nootkatin.

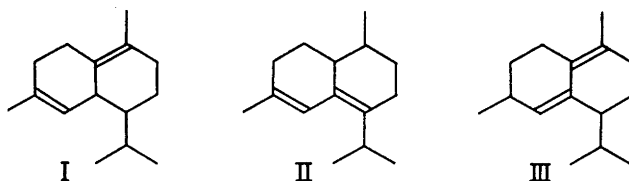
The non-volatile part of the extract from the wood of the common juniper (*Juniperus communis* L.) has been earlier investigated and found to contain several diterpene phenols¹⁻⁴. The steam volatile part was briefly investigated a few years ago⁵ and cedrene and cedrol were supposed not to occur in this material.

Through the courtesy of Professor and Mrs. Sörensen, Trondheim, a large sample of the steam volatile oil of the wood of *J. communis* was put at our disposal. The oil was extracted with alkali and the neutral part subjected to fractional distillation. The hydrocarbon fractions were analysed by gas chromatography. The main fraction was identified as thujopsene⁶. The fractions boiling below thujopsene were complex mixtures giving seven peaks in the gas chromatograms in addition to some minor ones. One of the major peaks (C₁) corresponded to that of the tricyclic sesquiterpene hydrocarbons Z and U from *Juniperus thurifera* L⁷. Another peak (E) corresponded to that of cedrene or longifolene (junipene) but neither compound could be identified.

The fractions in the boiling range 117-136°/9 mm gave ten peaks. By redistillation of the proper fractions and chromatography on alumina one substance could be isolated in a reasonably pure state and was identified as humulene by means of its infrared spectrum and that of its hydrogenation product^{8,9}. Similarly, a fraction giving a single peak on gas chromatography was obtained which on dehydrogenation with selenium furnished a large yield of cadalene and on treatment with hydrogen chloride a low yield of (-)-cadinene dihydrochloride. The infrared spectrum was, except for an additional band at 1 615 cm⁻¹ and some minor differences in the finger-print region, identical with that reported for δ -cadinene¹⁰. The ultraviolet spectrum, however,

showed a maximum at $248\text{ m}\mu$ (ϵ about 5 000). The freshly isolated fractions exhibited optical rotations ranging from -47° to -10° . These fractions were easily autoxidised and when the non-oxidised part of them was isolated it always had a lower negative rotation, sometimes even weakly positive.

From the above results this gas chromatographically homogeneous fraction appears to be a mixture of δ -cadinene and the *isocadinene*, reported to occur in small amounts in several oils together with δ -cadinene^{11,12}. The wood oil of the common juniper, however, seems to contain unusually large amounts of *isocadinene* that prevent the isolation of δ -cadinene in a pure state. The position of the UV absorption maximum ($248\text{ m}\mu$) for the *isocadinene* indicates that the double bonds are heteroannular and highly substituted. The infrared spectrum of the cadinene mixture possesses the same bands in the CH out-of-plane deformation range as pure δ -cadinene. These observations together with the structure proposed for δ -cadinene¹³ (I) enable the tentative structures II or III to be proposed for the *isocadinene*.



The presence of cuparene was indicated by gas chromatography and infrared spectra and pure cuparene could be isolated by ozonisation¹⁴. One peak (N) in the gas chromatograms was found to correspond to that of calamenene¹⁵ and the maxima at $1\ 500$ and $1\ 615\text{ cm}^{-1}$ in the IR spectra of the fractions giving this peak further supports¹¹ the assumption of the presence of this compound. It could, however, not be separated from cuparene.

The approximate amounts of the hydrocarbons, as percentage of the oil, calculated from the peak areas of the gas chromatograms, were as follows: "peak C₁" 1.5, "peak E" 1.5, thujopsene 37, humulene 4, δ -cadinene + *isocadinene* 12, cuparene 3, "peak N" 2. All the other peaks corresponded to compounds occurring in amounts less than 1 %. The amount of monoterpenes was very low, below 0.1 %.

The higher boiling fractions, containing alcohols and oxo-compounds, failed to crystallise. From the fractions, b.p. $142-147^\circ/9\text{ mm}$, cedrol and widdrol¹⁶ could be isolated by chromatography. The estimated amounts of cedrol and widdrol were 2 and 1 % of the oil, respectively. The alcohol, m.p. $75-76^\circ$, isolated during the previous investigation⁵ was not found in this oil but a direct comparison of the infrared spectra and a mixed m.p. kindly carried out by Dr. O. Motl, has shown it to be identical with the cadinol, m.p. 75° , from juniper berry oil¹⁷.

Oxo-compounds were isolated with Girard reagents from several of the "alcohol fractions". The infrared spectra of the products from the lower boiling fractions showed that they were complex mixtures and no crystalline semicarbazone could be obtained from them. From a high-boiling fraction a small

amount of a crystalline semicarbazone, $C_{16}H_{25}ON_3$, was obtained, m.p. 237–239° (decomp.). The infrared spectrum of the compound, m.p. 32–35°, obtained by hydrolysis of the semicarbazone and the ultraviolet spectrum of the semicarbazone indicated that the compound was an α,β -unsaturated aldehyde.

Zavarin, Smith and Anderson¹⁸ reported the presence of α -thujaplicin, nootkatin, pygmaein, and an unknown compound in a specimen of *Juniperus communis*, grown in California. The acidic fraction of our oil was therefore distilled but the distillates did not exhibit the spectral characteristics of tropolones. Since the oil investigated had been stored for a long time a sample of juniper wood was subjected to distillation with steam. The ultraviolet spectrum of the acidic fraction from the volatile products clearly showed the presence of tropolones and paper chromatography indicated the presence of α - and β -thujaplicin and nootkatin.

It has previously been suggested that cedrene and cedrol occur only in the Section *Sabina* of the junipers⁵. This, however, has now been shown to be incorrect since not only does *J. communis* contain cedrol but also *J. cedrus*¹⁹ and *J. rigida*²⁰, all belonging to the Section *Oxycedrus*. The amount of cedrol in the wood of the latter junipers is small, however, and it is a matter of some difficulty to isolate it.

EXPERIMENTAL

All m.p.'s were determined with a Kofler microscope and are corrected. The microanalyses were performed by Dr. A. Bernhardt, Mülheim. The infrared spectra were recorded with a Perkin-Elmer No. 21 instrument, and the ultraviolet spectra with a Beckman DK-2 instrument. The gas chromatograms were made on a Pye Argon Chromatograph and, when not otherwise stated, with a dinitrophenyl naphthyl ether column described elsewhere²¹. Light petroleum refers to b.p. 40–60°.

The juniper wood oil (4.1 kg), obtained in several batches had been stored for over ten years in an ice chest in well stoppered, dark flasks. The different batches, except one with the strongest negative rotation (I) were combined. The oil was extracted with 2 N NaOH and the acid extracts combined (13.5 g, 0.3 %). Two thirds (2.6 kg) of the combined neutral fractions were distilled in a column packed with metal spirals (diameter 5 cm, length 150 cm). All the following distillations were made in a 1 m LKB-column. Since the physical constants and relative amounts of the fractions in the distillation of the oil were essentially identical with the results earlier recorded⁵ they will not be mentioned here. The fractionation of the hydrocarbons was followed by gas chromatography and gave the following peaks, relative retention volumes, thujopsene = 1: several small peaks (monoterpenes) 0.09–0.22, A 0.50, B 0.61, C₁ 0.73, C₂ 0.78, D 0.85, E 0.89, F (thujopsene) 1, G 1.09, H 1.22, I 1.42, K 1.53, L 1.65, M 2.18, N 2.36, O 2.97, P 3.32.

Fractions b.p. 95–117°/9 mm. These fractions showed the peaks A–F. In the first fraction, b.p. 95–112°/9 mm, the peaks R_{rel} 0.09–0.22 were observed but only in a very low amount. They corresponded to monoterpenes. The fraction was yellow in colour. Upon chromatography on alumina neither the infrared nor the ultraviolet spectra of the yellow eluates showed the presence of thymoquinone, but a small amount of carvacrol methyl ether might have been present in this fraction. Some of the eluates had a strong odour of citronellol.

Peak C₁ corresponded to the peak obtained from the tricyclic sesquiterpene hydrocarbons Z and U from *J. thurifera*⁷. Peak E had the same retention volume as cedrene and longifolene. It always occurred together with peaks B, C, and D in small amounts in admixture with large amounts of peak F. Chromatography on alumina did not increase the relative amount of the substance corresponding to peak E but partial low-temperature ozonisation increased it slightly until an amount of about 12 % was attained. An infrared spectrum did, however, not show any of the bands of cedrene. This does not disprove the presence of cedrene since infrared spectra of known mixtures of thujopsene and

cedrene did not give any indication of the presence of cedrene when present in amounts below 15 %. Gas chromatograms of the E-enriched fractions were run on several different columns, the best results being obtained on Apiezon L. The results could not, however, give any decisive answer as to the presence or absence of either cedrene or longifolene. Peak F corresponded to thujopsene. One of the distillates, b.p. 116.5°/8 mm, n_D^{20} 1.5049, $[\alpha]_D^{20}$ -99° (oil), from batch I was gas chromatographically homogeneous. Its infrared spectrum was identical with that of thujopsene and selenium dioxide oxidation¹⁶ gave widdrenal, m.p. 74–75°, identified by mixed m.p. determination with authentic material.

Fractions b.p. 117–136°/9 mm. These fractions showed the peaks F–P. Attempts to isolate the substance corresponding to peak G by distillation and chromatography on alumina did not give the substance in a purity higher than 35 %. Peak I was more enriched in the fractions b.p. 122–126°/9 mm, which were redistilled. Chromatography with light petroleum on a hundredfold amount of alumina (Merck, nach Brockmann, activated at 170° for 24 h) of the distillates having the highest content of I gave the compound in the end fractions, a behaviour earlier found by Šorm *et al.*¹⁰ for humulene. The purest fraction obtained, n_D^{20} 1.5045, $[\alpha]_D^{20}$ -5.7° (CHCl_3 , c 10), had a purity of about 85 % (gas chromatography). Its infrared spectrum was identical with that of humulene⁸, except for a small band at 1525 cm^{-1} possibly due to the presence of small amounts of cuparene. Upon hydrogenation with PtO_2 in acetic acid the product consumed 3.1 moles H_2 /mole. The hydrogenated material was chromatographed on alumina giving an oil, n_D^{20} 1.4760, $[\alpha]_D^{20}$ -0.9° (CHCl_3 , c 10), purity about 85 % (gas chromatography). The infrared spectrum was in good agreement with the spectra published for humulene^{8,9}.

The compound corresponding to the peak K was enriched in the fractions b.p. 131–132°/9 mm. Chromatography on activated alumina gave in the first eluates gas chromatographically homogeneous "product" K. These eluates gave, upon selenium dehydrogenation, an 85 % yield of cadalene, picrate m.p. 116–117°, mixed m.p. with authentic material, no depression, and with hydrogen chloride in 20 % yield (–)-cadinene dihydrochloride, m.p. 117–118.5°, $[\alpha]_D^{20}$ -36.5° (CHCl_3 , c 2), mixed m.p. with authentic material, no depression. The very first eluate had a rotation of $[\alpha]_D$ -47° which fell to about -10° in the later fractions. All fractions had an ultraviolet maximum at 248 μ . The highest extinction value observed was ϵ 5700. The fractions were very sensitive to autoxidation, e.g. one fraction stored for a week in a refrigerator gave upon chromatography on alumina only one fourth of the original amount in the light petroleum eluate. The rotation had at the same time decreased from -28° to -4° .

Peak M corresponded to cuparene. A distillate of batch I with a low content of peak N (see below) was ozonised at low temperature¹⁴ and cuparene isolated by chromatography and distillation. The distillate, b.p. 117°/7 mm, n_D^{20} 1.5238, $[\alpha]_D^{20}$ $+67^\circ$ (CHCl_3 , c 2), was gas chromatographically homogeneous and the infrared spectrum was identical with that of cuparene. Peak N had the same retention volume as calamenene¹⁵. The fractions showing this peak had maxima in the infrared at 1615 and 1500 cm^{-1} . They could not be freed from cuparene.

Fractions b.p. 136–150°/9 mm. These fractions contained mainly alcohols. Upon fractionation with Girard D reagent small amounts of oxo-compounds were isolated. The infrared spectra showed that they were complex mixtures and no crystalline semicarbazones could be obtained from them. The carbonyl-free fractions were chromatographed on a fortyfold amount of alumina (Merck, nach Brockmann). Benzene-benzene + 1 % ether eluted crude cedrol, m.p. 45–70°. This was difficult to obtain pure by crystallisation, but gradient sublimation ultimately gave pure cedrol, m.p. 85–86°, $[\alpha]_D^{20}$ $+9^\circ$ (CHCl_3 , c 2), mixed m.p., IR. Benzene + ether (10:1 to 1:1) eluted widdrol, crystallised from acetonitrile, m.p. 97.5–98°, $[\alpha]_D^{20}$ $+103^\circ$ (CHCl_3 , c 2), mixed m.p., IR. No further crystalline compounds could be isolated.

In order to make sure that no other alcohols were present in the crude cedrol a preparation of m.p. 65–67° was subjected to dehydration with formic acid. The hydrocarbon fraction was isolated by chromatography on alumina and investigated by gas chromatography. It showed the cedrene peak as well as a few peaks due to very small

amounts of contaminating hydrocarbons. Similarly pure widdrol gave rise to a hydrocarbon mixture exhibiting three major peaks and four minor ones and α -, β -, δ - and *isocadinol* to a mixture showing thirteen peaks of different magnitude. The "impurity peaks" from cedrol cannot be correlated with certainty with any of these peaks.

Fractions b.p. 130–135°/2 mm. These fractions did not give any crystalline substance after chromatography. The benzene eluate from the fraction, b.p. 134–135°/2 mm, gave an infrared spectrum which indicated that this eluate probably contained a rather homogeneous oxo-compound. Fractionation with Girard D reagent of the whole fraction (13 g) gave 1.6 g of oxo-compounds from which a crystalline semicarbazone was obtained in the usual way. It gave, after crystallisation from ethanol, 0.30 g of leaflets, m.p. 237–239° (decomp.), $[\alpha]_D^{20}$ -31° (pyridine, *c* 2). λ_{\max} 271 $m\mu$ ($\log \epsilon$ 4.45). (Found: C 69.4; H 8.8; N 15.3. Calc. for $C_{16}H_{22}ON_2$: C 69.8; H 9.15; N 15.3.) From the mother liquors 0.30 g of crystals, $[\alpha]_D^{20}$ -25° , separated. The last crystals were steam distilled in the presence of phthalic anhydride yielding 0.14 g of steam-volatile oil which, after distillation, crystallised partly, m.p. 32–35°. The compound showed strong bands in infrared at 2 810, 2 720, 1 680, and 1 630 cm^{-1} (in CCl_4). Comparison of the spectrum with that of synthetic cedrenal showed that they were not identical.

Acidic fraction. The acidic fraction was distilled and ultraviolet spectra were taken from the distillates. No selective absorption was detected above 300 $m\mu$.

Steam distillation of wood. 1.13 kg milled juniper wood was steam distilled until 12 litres of distillate had been collected. Salt was added and the water exhaustively extracted with ether. The ether extract was shaken with 1 N NaOH and the acidic part (0.27 g, λ_{\max} 335 $m\mu$, a 9.2) recovered from the alkaline solution in the usual way. The ultraviolet spectrum of the neutral part exhibited no absorption above 300 $m\mu$. The acidic part was dissolved in 100 ml ether and extracted successively with 2 \times 50 ml of the following solutions: (A) 5 % $NaHCO_3$, saturated with CO_2 , (B) 5 % $NaHCO_3$, (C) 2.5 % $NaHCO_3$ + 2.5 % Na_2CO_3 , and (D) 5 % Na_2CO_3 , leaving a residue (E). Ultraviolet spectra and paper chromatograms²² were run on each fraction giving the following results: (A) 77 mg, no selective absorption, β -thujaplicin (+); (B) 20 mg, inflexion 310 $m\mu$ (a 2.8), β -thujaplicin (+); (C) 52 mg, max. 330 $m\mu$ (a 4.0), β -thujaplicin ++, α -thujaplicin (+); (D) 61 mg, max. 333 $m\mu$ (a 5.9), β -thujaplicin ++++, α -thujaplicin +, nootkatin +; (E) 58 mg, max. 337 $m\mu$ (a 23.2), β -thujaplicin +, α -thujaplicin +, nootkatin +++.

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