

## The Chemistry of the Natural Order Cupressales

### XXIX\*. Heartwood Constituents of *Juniperus thurifera* L.

JARL RONEBERG

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

The heartwood of *Juniperus thurifera* contains  $\alpha$ -cedrene, thujop-sene, two apparently new  $C_{15}H_{24}$  hydrocarbons, cuparene, cedrol, a compound  $C_{20}H_{16}O_6$ , a diol  $C_{30}H_{54}O_2$ , carvacrol, nootkatin,  $\beta$ -thujaplicin, hinokiic acid and "Widdringtonia acid II". Two further sesquiterpene hydrocarbons were present, which contained vinylidene groups; one of these probably has a curcumene skeleton.

In continuation of the studies at present being carried out in this laboratory on the heartwood constituents of the genus *Juniperus*<sup>1-3</sup>, an investigation was made of a sample of *Juniperus thurifera* obtained from Spain.

The wood was extracted with acetone and the light petroleum-soluble part of the acetone extract was separated into neutral and acid fractions. Crystallisation of the acidic material gave two sesquiterpene acids, hinokiic acid<sup>4</sup>, which has also been found in *J. chinensis*<sup>1</sup> and in *J. utahensis*<sup>2</sup>, and the "acid II" previously isolated from *Widdringtonia*<sup>5</sup>, from *J. chinensis*<sup>1</sup> and from several other species of the *Cupressaceae*.

The acidic material was treated with copper acetate to precipitate any tropolones present. The product obtained on decomposition of the precipitate with hydrochloric acid gave nootkatin; according to paper chromatography it also contained  $\beta$ -thujaplicin. Paper chromatography showed the presence of carvacrol in the remaining acidic material.

The neutral oil was distilled. The lowest boiling part of the main sesquiterpene fraction was chromatographed on polyvinyl chloride powder impregnated with light petroleum, using a solution of silver fluoborate in aqueous methanol as eluant. This gave five main fractions x, y, z, u and v. Compounds containing vinylidene groups would be expected to form relatively strong silver complexes and therefore to be eluted first. In accordance with this the infrared spectrum of the first fraction x showed strong absorption at 1 635, 1 645 and 883  $cm^{-1}$  indicating the presence of vinylidene groups.

\* Part XXVIII. *Acta Chem. Scand.* 14 (1960) 1288.

Gas chromatographic analysis showed that fraction x contained two components ( $X_1$  and  $X_2$ ) in about equal amounts. The retention time ( $R_t$ ) of  $X_1$  was close to that of  $\alpha$ -cedrene and that of  $X_2$  was close to that of thujopsene. It was thus impossible to detect the compounds  $X_1$  and  $X_2$  by gas chromatography with the system used when  $\alpha$ -cedrene and thujopsene were present.

On dehydrogenation with selenium,  $X_2$  gave dihydro-*ar*-curcumene, previously obtained in the same way from "Substance Y" of a crude sesquiterpene hydrocarbon fraction isolated from *Juniperus virginiana*<sup>3</sup>. Gas chromatography showed that  $X_1$  remained almost unchanged after similar treatment with selenium.

Fraction y was pure thujopsene and on ozonolysis gave the corresponding keto-carboxylic acid<sup>4</sup>.

Fractions z and u gave two sesquiterpene hydrocarbons Z and U which, apparently, have not been isolated previously. According to elemental analysis and infrared spectra both compounds may be tricyclic with one double bond. On gas chromatography both had almost the same  $R_t$ -value but were easily separated from  $\alpha$ -cedrene and thujopsene. The  $R_t$ -values of Z and U agree well with the  $R_t$ -value of peak  $x_3$  on a gas chromatogram of a crude sesquiterpene hydrocarbon fraction from *Juniperus virginiana*<sup>3</sup>. A sample of U was dehydrogenated with selenium and the product was chromatographed but no crystalline picrate could be obtained.

Fraction v was  $\alpha$ -cedrene and gave on oxidation  $\alpha$ -cedrenic acid<sup>6</sup>.

The sesquiterpene hydrocarbon fraction, b.p. 121—123°/10 mm, showed aromatic absorption in the ultraviolet. It was redistilled and ozonised at low temperature. The material unaffected by ozone was identified as cuparene<sup>7</sup>.

Chromatography of the sesquiterpene alcohol fraction gave cedrol but no widdrol<sup>5</sup> was detected. This fraction, however, also gave a mixture of carbonyl compounds.

The highest boiling neutral oil afforded a small amount of crystalline material. On chromatography this was separated into three compounds:

The waxy compound I, m.p. 67.5—68.5° was not further investigated.

Compound II, m.p. 156.0—157.5° analysed for  $C_{20}H_{16}O_6$  and contained no methoxyl group, its ultraviolet spectrum showed a shoulder at 220  $m\mu$  ( $\epsilon$  19 200) and maxima at 237  $m\mu$  ( $\epsilon$  19 030), 293  $m\mu$  ( $\epsilon$  16 600) and 333  $m\mu$  ( $\epsilon$  22 300). The infrared spectrum showed aromatic absorption (1 495, 1 507, 1 606 and 1 625  $cm^{-1}$ ) and appeared to indicate the presence of a lactone ring (1 745  $cm^{-1}$ ). In addition there was a medium strength absorption peak at 1 644  $cm^{-1}$ . A small amount of a substance now shown to be identical with "compound II" has previously been isolated from *J. communis*<sup>8</sup>. This compound is presently under investigation.

Compound III, m.p. 243.5—244.0° analysed for  $C_{30}H_{54}O_2$ . It appeared to contain two hydroxyl groups; an acetate was prepared but it gave a poor analysis.

The compounds isolated are listed below with very approximate estimates of the amounts present (as percentages of the air-dried wood). Total acetone extract 9.9, ether-soluble acetone extract 7.3, light petroleum-soluble acetone extract 5.7, sodium bicarbonate-soluble 0.03, potassium hydroxide-soluble 1.0, neutral 4.6, nootkatin 0.01,  $\beta$ -thujaplicin 0.001, carvacrol 0.1, hinokiic acid

0.2, "Widdringtonia acid II" 0.07, sesquiterpene hydrocarbons  $X_1 + X_2$  0.3, sesquiterpene hydrocarbon Z 0.01, sesquiterpene hydrocarbon U 0.05,  $\alpha$ -cedrene 0.6, thujopsene 0.4, cuparene 0.1, cedrol 0.7, "compound II" 0.005, "compound III" 0.003, high-boiling carbonyl compounds 0.03.

### EXPERIMENTAL

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

*Isolation of the oils.* Ground, air-dried heartwood (15.2 kg) of *J. thurifera* was extracted with acetone for 24 h. The acetone was evaporated and the oil obtained was poured with stirring into ten volumes of ether. The ether solution was filtered (precipitate A, 388 g), evaporated and the residue was poured into ten volumes of light petroleum. After filtration (precipitate B, 254 g) the solution was evaporated to dryness, diluted with two volumes of light petroleum and extracted successively with sodium bicarbonate (saturated solution) and potassium hydroxide (2 N). Acidification of the alkaline solutions and extraction with ether gave a sodium bicarbonate-soluble fraction C (4.4 g), which was not further investigated, and a potassium hydroxide-soluble fraction D (152 g). The remaining light petroleum solution was washed with water, dried and evaporated giving neutral material E (704 g).

*Alkali-soluble fraction.* The potassium hydroxide-soluble oil D was diluted with two volumes of ether and extracted with sodium carbonate-solution (2 N). On acidification and extraction with ether an oil F (124 g) was obtained, which partly crystallised on standing. The material insoluble in sodium carbonate was an oil G (24 g).

The crystalline part (41.4 g) of F was filtered off and a sample (3 g) was fractionally recrystallised from ether. The material less soluble in ether (0.9 g) was recrystallised from light petroleum, m.p. 190.0–191.5°,  $[\alpha]_D - 99^\circ$  (c. 1.5), and identified (mixed m.p., IR) as the "acid II", isolated from *Widdringtonia* <sup>6</sup>. The material slightly more soluble in ether, m.p. 166.0–167.5°, was identified as *hinokiic acid* <sup>4</sup>.

The oil G (24.0 g) was dissolved in five volumes of ligroin (b.p. 100–125°) and shaken with aqueous copper acetate (saturated solution). The copper salts were filtered off and washed with ligroin and water to give a combined ligroin phase H. The salts were decomposed with hydrochloric acid (2 N) and the resulting product was extracted with ether. The residue obtained on evaporation of the ether solution (2.4 g) was recrystallised from light petroleum and sublimed in a high vacuum, m.p. 93.5–95.0°. It was identified as *nootkatin* (mixed m.p., IR). The presence of  $\beta$ -*thujaplicin* in the mother liquors was demonstrated by paper chromatography <sup>9</sup>.

The ligroin phase H was separated, washed with water and evaporated to dryness. The residue was shown by paper chromatography <sup>9</sup> to contain *carvacrol*.

*Neutral fraction.* The neutral oil E (518 g) was subjected to a quick preliminary distillation up to 150°/2 mm giving a distillate K (476 g) and a high-boiling residue L (43 g). A sample of the neutral oil K was distilled through a one meter, vacuum-jacketed, packed column giving the fractions listed in Table i.

Fraction Ib was redistilled through a 70 cm spinning band column and the first fraction (IIa, 3.0 g), b.p. 136–137°/25 mm,  $[\alpha]_D - 42^\circ$ ,  $n_D^{25}$  1.4975 was chromatographed according to a method developed by B. Wickberg using polyvinylchloride powder impregnated with light petroleum and eluting with a solution of silver fluoborate in aqueous methanol. A detailed account of the separation will be published later in this journal. The main fractions, x, y, z, u and v, were eluted in this order. Fraction x (279 mg) b.p. 121°/10 mm was a mixture of two compounds  $X_1$  and  $X_2$  which both, according to the infrared spectrum, appear to contain vinylidene groups. The ultraviolet spectrum of the fraction (in 95% ethanolic solution) showed maxima at 205, 206 and 242  $\mu$ .

*Thujopsene.* Fraction y (314 mg) was redistilled and a sample (200 mg) in ethyl acetate-methanol (4:1, 10 ml) was ozonised at –70° until the blue colour indicating excess ozone appeared. When the reaction mixture had reached room temperature zinc dust (1 g) activated with acetic acid was added. After one hour, ether (50 ml) was added

Table 1. Distillation of neutral fraction K (256 g). Total distillate 212 g or 83 %.

| Fraction | Weight<br>(g) | B.p.<br>(°C) | Pressure<br>(mm Hg) | Rotation<br>[ $\alpha$ ] <sub>D</sub> | Refractive<br>index ( $n_D^{25}$ ) |
|----------|---------------|--------------|---------------------|---------------------------------------|------------------------------------|
| Ia       | 9.5           | 64–126       | 14                  | –39                                   | 1.4968                             |
| Ib       | 68.3          | 126–127      | 14                  | –68                                   | 1.4993                             |
| Ic       | 47.3          | 127–149      | 14                  | –14                                   | 1.5078                             |
| Id       | 10.1          | 149–158      | 14                  | +15                                   | –                                  |
| Ie       | 28.0          | 158          | 14                  | +14                                   | –                                  |
| If       | 25.1          | 158–160      | 14                  | +16                                   | –                                  |
| Ig       | 11.4          | 160–163      | 14                  | +23                                   | –                                  |
| Ih       | 12.3          | 142–145      | 3                   | +22                                   | 1.5145                             |

followed by aqueous acetic acid (10 %, 50 ml); the reaction mixture was filtered and the organic phase was separated, washed with water, dried and evaporated to dryness. The residue (0.65 g) was recrystallised from ethyl acetate, m.p. 165.0–166.0°, and identified as thujopsene ketocarboxylic acid <sup>4</sup> (mixed m.p., IR).

Fraction z (73 mg, from 6 g of IIa) was redistilled to give *sesquiterpene hydrocarbon Z*, b.p. 122°/10 mm, [ $\alpha$ ]<sub>D</sub> + 75° (c, 2.2),  $n_D^{25}$  1.4942. (Found: C 88.2; H 11.8; CH<sub>3</sub>-(C) 5.6. C<sub>15</sub>H<sub>24</sub> requires C 88.2; H 11.8; CH<sub>3</sub>-(C) (one) 7.4.) Infrared spectrum (in carbon tetrachloride solution): 3 010 s, 2 960 vs, 2 870 vs, 2 720 w, 1 640 w, 1 462 vs, 1 450 vs, 1 433 vs, 1 375 vs, 1 364 vs, 1 346 m, 1 327 w, 1 315 m, 1 296 m, 1 279 s, 1 186 s, 1 175 m, 1 154 s, 1 129 s, 1 102 w, 1 062 m, 1 045 w, 1 021 s, 958 w, 945 w, 925 m, 910 cm<sup>-1</sup>. The ultraviolet spectrum of this fraction (in 95 % ethanolic solution) showed a maximum at 207 m $\mu$  ( $\epsilon$  1 050).

Fraction u (298 mg, from 6 g of IIa) was redistilled to give *sesquiterpene hydrocarbon U*, b.p. 122°/10 mm, [ $\alpha$ ]<sub>D</sub> + 33° (c, 2.2),  $n_D^{25}$  1.4961. (Found: C 88.0; H 11.7; CH<sub>3</sub>-(C) 6.5; M 209 (Rast). C<sub>15</sub>H<sub>24</sub> requires C 88.2; H 11.8; CH<sub>3</sub>-(C) (one) 7.4; M 204.) Infrared spectrum (pure oil): 3 065 s, 2 950 vs, 2 745 w, 1 647 w, 1 485 s, 1 467 vs, 1 448 vs, 1 390 s, 1 378 vs, 1 366 vs, 1 341 m, 1 330 m, 1 320 m, 1 295 w, 1 280 w, 1 223 w, 1 211 w, 1 194 w, 1 175 m, 1 134 w, 1 098 w, 1 083 w, 1 063 w, 1 035 w, 1 023 m, 1 005 m, 983 w, 972 m, 925 s, 909 w, 867 w, 839 m, 794 s, 777 s, 695 cm<sup>-1</sup>.

A sample of fraction x (52 mg) was heated with selenium (50 mg) in a carbon dioxide atmosphere for 24 h at 270°. A gas chromatogram showed that the compound X<sub>1</sub> was largely unchanged; no peak corresponding to X<sub>2</sub> could be detected but a new peak was obtained with the same R<sub>f</sub>-value as dihydro-*ar*-curcumene <sup>3</sup>. The dehydrogenation product was ozonised as described for cuparene (see below) and the material unattacked by ozone (20 mg),  $n_D^{25}$  1.4865, was identified as dihydro-*ar*-curcumene by comparison of its infrared spectrum with that of a synthetic sample <sup>10,11</sup>.

*$\alpha$ -Cedrene*. Fraction v (1.47 g, from 3 g of IIa) was redistilled, b.p. 113°/11 mm, [ $\alpha$ ]<sub>D</sub>-86° (c, 2.8),  $n_D^{25}$  1.4960. A sample (0.78 g) in ethanol (95 %, 15 ml) was refluxed with selenium dioxide (0.47 g) for 3 h. The reaction product was filtered, the alcohol-solution was evaporated to dryness and the residue was distilled under reduced pressure. The distillate (0.54 g) was added to a suspension of silver oxide (sodium hydroxide (0.38 g), silver nitrate (0.79 g)) in water (60 ml) and the mixture was refluxed with vigorous stirring for 9 h. The solution was then filtered, and after extraction with ether to remove neutral material, the alkaline solution was acidified and extracted with ether. This ether extract was evaporated and the product (0.26 g) was sublimed three times in a high vacuum giving a substance, m.p. 123.0–124.5°, which was identified as  $\alpha$ -cedrenic acid <sup>4</sup> (mixed m.p., IR).

*Cuparene*. Fraction Ic was redistilled through a 70 cm spinning band column and the fractions obtained were analysed by gas chromatography (*cf.* below). The material (3.0 g) containing most cuparene was dissolved in methylene chloride (15 ml) and treated at –70° with an excess of ozone. When the reaction mixture had reached room temperature, hydro-

gen peroxide (30 %, 8 ml) in potassium hydroxide (10 %, 3 ml) was added and the organic phase was separated, washed with water and evaporated giving a viscous oil. This was chromatographed on basic alumina (40 g). The first 100 ml of light petroleum eluted a hydrocarbon (0.13 g) which was distilled under reduced pressure, b.p. 117°/7 mm,  $[\alpha]_D + 63^\circ$  (c, 2.0),  $n_D^{25}$  1.5207, and identified (IR) as cuparene<sup>7</sup>.

*Gas chromatographic investigation of the sesquiterpene hydrocarbon fractions.* Gas chromatograms were run on a Pye Argon Chromatograph using a system described in a previous paper in this series<sup>3</sup>. (Temp. 150°, charge 0.025  $\mu$ l, argon flow rate 27 ml/min). This gave the following retention times (min): Compound Z 13.2, compound U 13.7, compound X<sub>1</sub> 17.3,  $\alpha$ -cedrene 17.4, thujopsene 19.1, compound X<sub>2</sub> 20.2, dihydro-*ar*-curcumene 21.6 and cuparene 42.8.

*Cedrol.* The crystalline part (1.36 g) of Ig was separated by filtration (filtrate Ig<sub>2</sub>) and a sample (300 mg) was chromatographed on basic alumina (50 g). Ether-benzene (1:5) eluted a substance (210 mg) which was recrystallised from ethanol (95 %), m.p. 88.5–89.5°,  $[\alpha]_D + 11^\circ$ , (c, 1.9); this was identified as cedrol (mixed m.p., IR). Ether-benzene (1:1) and ether eluted small amounts of an oil with an infrared spectrum different from that of widdrol<sup>8</sup>.

*High-boiling carbonyl compounds.* Fraction Ig<sub>2</sub> (5.5 g) in ethanol (95 %, 20 ml) containing acetic acid (10 %) was refluxed with Girard D reagent (2.0 g) for 30 min and the reaction product was extracted with ether at 0°. The aqueous solution was acidified with an equal volume of hydrochloric acid (4 N) while kept cool and then extracted with ether. The ether solution yielded a liquid mixture of carbonyl compounds (0.88 g); these were not further investigated.

The high-boiling neutral residue L, on standing, deposited a small amount of crystalline material. This was filtered off (1.49 g) and recrystallised from benzene to give compound III (240 mg, see below). The mother liquor was evaporated to dryness and the residue (1.25 g) was chromatographed on silica gel (200 g).

*Compound I.* Ether-benzene (1:1.7) eluted a substance (93 mg) which was recrystallised from ethanol (95 %) giving compound I, m.p. 67.5–68.5°,  $[\alpha]_D - 2^\circ$  (c, 1.3). (Found: C 78.3; H 12.7.)

*Compound II.* Ether-benzene (3:7) eluted a yellowish compound, m.p. 141–144°,  $[\alpha]_D - 75^\circ$  (c, 1.4). On recrystallisation from benzene and then from ethanol (95 %) followed by sublimation in a high vacuum the compound melted at 146–150°,  $[\alpha]_D - 20^\circ$  (c, 2.9) and two further sublimations gave colourless crystals, m.p. 156.0–157.5°,  $[\alpha]_D - 4^\circ$  (c, 0.7). (Found: C 68.5; H 4.7; CH<sub>3</sub>O– 0.0; CH<sub>3</sub>–(C) 2.5; M (Rast) 346. C<sub>20</sub>H<sub>18</sub>O<sub>6</sub> requires C 68.2; H 4.6; CH<sub>3</sub>–(C) (one) 4.3; M 352.) Compound II in methanol gave no colour with methanolic ferric chloride. On attempted acetylation with acetic anhydride and pyridine at 100° for 5 h most of the starting material was recovered unchanged. Compound II did not react with 2,4-dinitrophenylhydrazine in dilute hydrochloric acid and was recovered unchanged when treated for 24 h with a mixture of diazomethane in ether and methanol.

*Compound III.* Ether-benzene (7:3) eluted a compound (143 mg) which was combined with the sample of compound III obtained above. The combined material was recrystallised from benzene and then from ethanol (95 %) and sublimed in a high vacuum, m.p. 243.5–244.0°,  $[\alpha]_D + 14^\circ$  (c, 2.2). (Found: C 80.3; H 11.9; active H 0.43; CH<sub>3</sub>–(C) 9.4. C<sub>30</sub>H<sub>54</sub>O<sub>2</sub> requires C 80.6; H 12.2; active H (one) 0.22; CH<sub>3</sub>–(C) (one) 3.4.) Infrared spectrum (KBr): 3 430 s, 2 935 vs, 2 880 vs, 2 660 w, 1 633 w, 1 465 s, 1 450 s, 1 386 s, 1 370 m, 1 336 m, 1 304 w, 1 285 m, 1 270 m, 1 252 m, 1 235 m, 1 211 w, 1 166 m, 1 140 w, 1 120 w, 1 075 m, 1 050 w, 1 035 m, 1 005 s, 974 s, 963 m, 945 w, 902 w, 880 w, 870 w, 845 w, 826 w, 730 w, 702 cm<sup>-1</sup> w.

Compound III on acetylation (acetic anhydride/pyridine) afforded an acetate, m.p. 127.0–128.0°. (Found: C 76.1; H 10.8. C<sub>34</sub>H<sub>58</sub>O<sub>4</sub> requires C 76.9; H 11.0.)

*Acknowledgements.* My thanks are due to Professor H. Erdtman for his interest in this work, to Ingeniero José Benito Martínez, Instituto Forestal, Madrid, Spain, for his kind assistance in obtaining the wood sample, to Miss G. Hammarberg for infrared and ultraviolet spectra and to the Swedish Natural Science Research Council for financial support.

## REFERENCES

1. Runeberg, J. *Acta Chem. Scand.* **14** (1960) 353.
2. Runeberg, J. *Acta Chem. Scand.* **14** (1960) 797.
3. Runeberg, J. *Acta Chem. Scand.* **14** (1960) 1288.
4. Erdtman, H. and Norin, T. *Acta Chem. Scand.* **13** (1959) 1124.
5. Erdtman, H. and Thomas, B. R. *Acta Chem. Scand.* **12** (1958) 267.
6. Treibs, W. *Ber.* **70** (1937) 2066.
7. Enzell, C. and Erdtman, H. *Tetrahedron* **4** (1958) 361.
8. Bredenberg, J. B. *Private communication.*
9. Wachtmeister, C. A. and Wickberg, B. *Acta Chem. Scand.* **12** (1958) 1335.
10. Batt, R. D. and Slater, S. N. *J. Chem. Soc.* **1949** 834.
11. Ruzicka, L. and van Veen, A. G. *Ann.* **468** (1929) 143.

Received May 23, 1960.