

**The Chemistry of the Natural Order Cupressales**  
**XXIV.\* Heartwood Constituents of *Chamaecyparis thyoides***  
**(L) Britt.**

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The heartwood of *Chamaecyparis thyoides* was found to contain carvacrol methyl ether,  $\alpha$ -cedrene, cuparene, thujopsene (widdrene), cedrol, widdrol, cuparenic acid, hinokiic acid (widdrenic acid) and "Widdringtonia acid II"; no tropolones were detected.

The family *Cupressaceae* has been subdivided by botanists into about twenty genera, most of which are monotypic or include only a few species. Both chemical and botanical aspects of the relationship between the different genera and the various species of the larger genera have been discussed in a recent review<sup>1</sup>.

The present investigation was undertaken to provide a chemical characterization of *Chamaecyparis thyoides*, a tree endemic to the south-eastern part of North America. Zavarin and Anderson<sup>2</sup>, in a survey of the occurrence of tropolones, have previously examined an extract from this tree by paper chromatography and have reported the presence of  $\alpha$ -,  $\beta$ - and  $\gamma$ -thujaplicins.

The heartwood of *Chamaecyparis thyoides*, very kindly provided by Dr. J. P. Bain, Glidden Co., Jacksonville, Florida, was extracted with acetone and the light petroleum-soluble part of this extract was separated into acidic and neutral fractions. The neutral part, after a preliminary distillation, was fractionated through a high efficiency column giving a forerun, which contained mainly carvacrol methyl ether, and three main fractions with practically constant boiling points.

The first fraction was a mixture of sesquiterpene hydrocarbons, which according to gas chromatographic examination contained two main components in about equal amounts. Oxidation of the hydrocarbon mixture with selenium dioxide furnished an oil, from which widdrenal<sup>3</sup> was isolated in crystalline form. The non-crystalline material on further oxidation with silver oxide and chromatography on dimethyl sulphoxide-impregnated silica gel gave cedrene carboxylic acid and hinokiic acid. The oxidation product of widdrenal, "widdrenic acid"<sup>3</sup>, has recently been shown<sup>4</sup> to be identical with hinokiic

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acid. The hydrocarbon fraction thus contains  $\alpha$ -cedrene and thujopsene. The optical rotation and the infra-red absorption ( $1647$  and  $885\text{ cm}^{-1}$ ) of this fraction were, however, consistent with the presence of a minor constituent of more positive rotation containing a  $\text{RR}'\text{C}=\text{CH}_2$ -group, possibly the so-called  $\beta$ -cedrene<sup>5</sup>.

Hirose and Nakatsuka<sup>6</sup> have shown that the "thujopsene" originally isolated by Kawamura<sup>7</sup> from *Thujopsis dolabrata* (Linneus, f) Sieb. et Zucc., on oxidation with selenium dioxide furnished a crystalline aldehyde which was identical with the widdrenal previously obtained by Erdtman and co-workers<sup>3,8</sup> from "widdrene", a hydrocarbon isolated from several *Widdringtonia* species and from *Biota orientalis*. Recently Erdtman and Norin<sup>4</sup> obtained pure "widdrene" from *Thujopsis dolabrata* and suggested that the name thujopsene should be retained for this hydrocarbon. Although it was clear that several of the oils containing thujopsene also contained a second component this had not been identified. It now seems probable that many of these oils are mixtures of thujopsene and  $\alpha$ -cedrene and that similar mixtures are of frequent occurrence.

Sesquichamene (b.p.  $122\text{--}123^\circ/12\text{ mm}$ ,  $d_4^{28}$   $0.9277$ ,  $n_D^{28}$   $1.5021$ ,  $[\alpha]_D^{28}$   $-89.85^\circ$ ), isolated by Kafuku and Nozoe<sup>9</sup> from the leaves of *Chamaecyparis obtusa*, Sieb. et Zucc. and later by Katsura<sup>10</sup> from the leaves of *Chamaecyparis formosensis*, Mats. on oxidation with potassium permanganate, gave a glycol  $\text{C}_{15}\text{H}_{26}\text{O}_2$ , m.p.  $89\text{--}91^\circ$ , and a ketoaldehyde,  $\text{C}_{14}\text{H}_{20}\text{O}_2$ , disemicarbazone m.p.  $233^\circ$ . It has been suggested<sup>11</sup> that sesquichamene is identical with  $\alpha$ -cedrene, but the physical constants of sesquichamene and the similarity of the melting points of the oxidation products to those of thujopsene glycol<sup>12</sup> ( $92\text{--}93^\circ$ ) and cedrene ketoaldehyde disemicarbazone ( $233^\circ$ ) makes it probable that it was in fact a mixture containing mainly thujopsene and  $\alpha$ -cedrene.

The main component of the second fraction from *Chamaecyparis thyoides* was the aromatic sesquiterpene hydrocarbon cuparene. The isolation of this compound and the elucidation of its structure have been described in a previous paper<sup>13</sup>.

The third fraction was a crystalline mixture of the sesquiterpene alcohols cedrol and widdrol<sup>3</sup>, which were separated by chromatography on alumina. Widdrol is apparently frequently associated with cedrol<sup>3,14</sup>; its structure is at present being investigated.

On standing, the acidic fraction from *Chamaecyparis thyoides* deposited crystalline material, which after recrystallisation gave pure "Widdringtonia acid II"<sup>3</sup>, m.p.  $191\text{--}193^\circ$ . The remainder of the material on distillation gave a small amount of low-boiling forerun and a higher-boiling, crystalline main fraction. According to paper chromatographic results, the low-boiling material contained at least five components, but no tropolones and no carvacrol were detected. The crystalline main fraction gave hinokiic acid on crystallisation and the remaining mixture was separated into hinokiic acid and cuparenic acid<sup>13</sup> by chromatography on dimethyl sulphoxide-impregnated paper.

The absence of tropolones from the sample of wood examined in this investigation is not in accordance with the results obtained by Zavarin and Anderson<sup>2</sup>, but this divergence is probably due to the variations not infrequent in biological material.

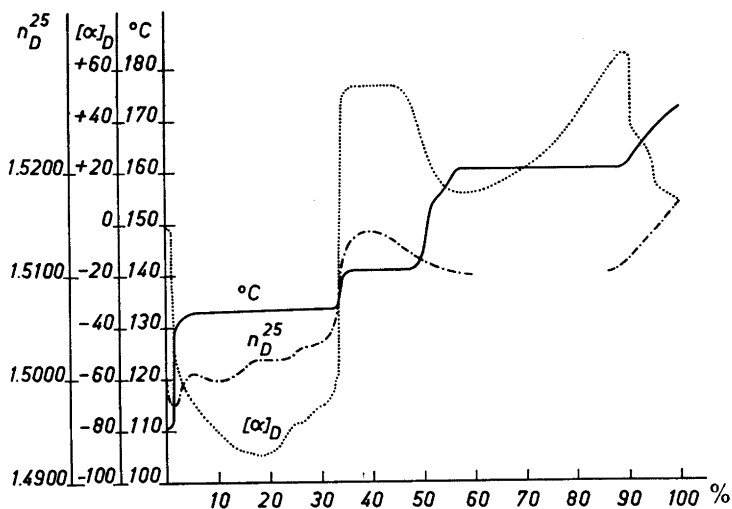


Fig. 1. Distillation of the main neutral fraction, — b. p. ( $^{\circ}\text{C}/20$  mm), .....  $[\alpha]_D$ , - - - - -  $n_D^{25}$ .

The results of the present study show a pattern of heartwood constituents which is remarkably similar to that of the Southern hemisphere genus *Widdringtonia*<sup>3</sup>. Any detailed comparison with the patterns of heartwood constituents of other species of *Chamaecyparis* and with those of other genera<sup>2</sup> of the Cupressaceae is however impeded by the lack of sufficient data<sup>1</sup>.

#### EXPERIMENTAL

Melting points were taken on a Kofler micro hot stage (uncorr). Unless stated to the contrary, rotations were measured in chloroform. Infrared spectra were recorded on a Perkin-Elmer No 21 instrument (NaCl-prism; liquids, pure oils in an 0.025 mm NaCl-cell). Light petroleum refers to a fraction b.p. 35–60°, unless otherwise stated. Microanalyses by Dr. A. Benhardt, Mülheim.

**Extraction.** Finely milled air-dry heartwood of *Chamaecyparis thyoides* (9.9 kg) was extracted with acetone for 48 h. The extract was filtered, concentrated to small volume and poured with vigorous stirring into a tenfold volume of ether. The precipitate (25 g) was filtered off, washed with ether and set aside.

The combined ether solutions were concentrated and poured into light petroleum in the same way. This precipitate (50 g) after extraction with light petroleum was also set aside. The light petroleum solution was concentrated to one litre, extracted successively with aqueous potassium hydroxide (5 % and then 10 %) and washed with water. The alkaline solutions were combined, washed with light petroleum, acidified with dilute sulphuric acid (10 %) and extracted with light petroleum. The combined neutral solution and the acidic solution gave, after drying ( $\text{Na}_2\text{SO}_4$ ) and evaporation of the solvent, a neutral fraction, a yellow oil (340 g, 3.5 % of the wood), and an acidic fraction, a redbrown oil (37 g, 0.4 % of the wood).

The neutral oil (340 g) was distilled to give a fraction, b.p. 120°/50 mm–180°/6 mm (260 g). The residue (80 g) gave two crystalline compounds in very small amounts, a "C<sub>15</sub>-ketone", m. p. 108–109°,  $[\alpha]_D +73^{\circ}$ , 2,4-dinitrophenylhydrazone, m. p. 198–199° (Found: N 14.2. C<sub>21</sub>H<sub>22</sub>N<sub>4</sub>O<sub>4</sub> requires N 14.5), and a substance, m. p. 140–141°,  $[\alpha]_D +58^{\circ}$  (Found: C 68.3; H 10.4. C<sub>21</sub>H<sub>38</sub>O<sub>5</sub> requires C 68.1; H 10.3), but it has not yet been further investigated.

The main part of the distillate was fractionally distilled through a vacuum-jacketed, packed column and where necessary refractionated through a spinning band column.

Curves for the distillation of the main neutral fraction showing boiling point (20 mm), refractive index and rotation plotted against percentage of material distilled are given in Fig. 1. There were three main fractions and a small forerun.

The forerun (0 → 1 %), according to its infrared absorption, contained mainly carvacrol methyl ether. Demethylation with hydrogen bromide (30 %, 1.5 h) followed by etherification with ethyl chloroacetate and saponification of the ester gave carvacroxy-acetic acid (50 %), m. p. and mixed m. p. 152–153°.

The fraction b. p. 131–133° (2 → 32 %) was a mixture of sesquiterpene hydrocarbons. A gas chromatogram on a Perkin-Elmer vapour fractometer model 154 B (two metre column, 2,4-dinitrophenyl-2-naphthyl ether (20 %) on celite; column temp. 156°; flow rate 50 ml/min of helium; charge 5  $\mu$ l) showed two peaks with elution times of 14 and 15 min and percentage of total area 48 and 52, respectively. This mixture (2.0 g) was oxidised with selenium dioxide (1.2 g) in ethanol (30 ml) for 3 h at reflux temperature. Evaporation of the solvent followed by repeated distillation of the product and recrystallisation from light petroleum gave widdrenal (0.2 g), m. p. and mixed m. p. 75–76°; infrared spectrum identical with that of authentic material. The non-crystalline part of the oxidation product (1.1 g) was added to a suspension of silver oxide (silver nitrate, 1.6 g, and sodium hydroxide 0.8 g) in water (15 ml) and the mixture was stirred vigorously for 2 h at 100°. The reaction mixture was separated into a neutral fraction (0.4 g) and an acid fraction (0.6 g). The chloroform-soluble part (0.2 g) of the acid fraction was chromatographed on dimethyl sulphoxide-impregnated silica gel.

This dimethyl sulphoxide-impregnated silica gel column was prepared by a method developed by Lindberg and Wickberg<sup>15</sup> for the separation of acetylated sugars. Dried, acid-washed silica gel (0.03–0.08 mm, 30 ml) was suspended in a solution of dimethyl sulphoxide in chloroform (5 %, 100 ml). The suspension was kept under reduced pressure in a vacuum desiccator for a short time and then transferred to a separatory funnel fitted on the top of a chromatogram tube that was completely filled with the same chloroform solution. Moderate pressure was applied to the top of the separatory funnel and the taps were opened to allow the suspension to replace the solution in the tube. The silica gel (1.5 × 24 cm) was saturated with dimethyl sulphoxide by passing a solution of dimethyl sulphoxide in ether (5 %, 150 ml). This was followed by a small volume of pure ether (5 ml), to prevent precipitation of dimethyl sulphoxide by the eluant to be used, and finally by the eluant (100 ml).

The acid mixture (0.2 g) was absorbed on the column and eluted with a mixture of light petroleum (b. p. 60–71°) and isopropyl ether (2:1, 6 ml fractions). The separation was most conveniently followed by the test chromatograms of the eluate on dimethyl sulphoxide-impregnated paper<sup>16</sup> with light petroleum and using a mixed indicator as spray reagent. The acids were found in fractions 15–18 and 20–26. The first acid (0.1 g) after sublimation had m. p. 122–124°,  $[\alpha]_D -68^\circ$  (c 2.2) (cf. authentic cedrenecarboxylic acid, m. p. 122°). Cedrol (8.4 g) was dehydrated with formic acid, refluxed with methanolic potassium hydroxide (10 %, 0.5 h) and filtered through alumina (200 g) in light petroleum to give  $\alpha$ -cedrene<sup>16</sup> (7.0 g),  $[\alpha]_D -91^\circ$  (pure oil),  $d_{20}^{20}$  0.9371,  $n_D^{25}$  1.4968. It was gas chromatographically homogeneous (conditions as above, elution time 14 min) and the infrared spectrum indicated that there was no exocyclic methylene group present.

$\alpha$ -Cedrene (4 g) was oxidised with selenium dioxide in ethanol to give cedrenal<sup>17</sup> (3.8 g) and a part (0.6 g) of this aldehyde was treated with an aqueous silver oxide suspension at reflux temperature for 15 h giving cedrene carboxylic acid<sup>17</sup> (0.3 g). The infrared spectra of the two acids were superimposable and the mixed m. p. was undepressed, 122–124°. The second acid (0.1 g), m. p. 169–170°, was identified as hinokiic acid, mixed m. p. undepressed, infrared spectra identical.

The fraction b. p. approx. 141° (34 → 47 %) after purification as described in a previous paper<sup>13</sup> gave pure cuparene (b. p. 138°/19 mm,  $d_{21}^{21}$  0.9374,  $[\alpha]_D +65^\circ$ ,  $n_D^{25}$  1.5202) in 45 % yield. It was further characterised by oxidation with chromic acid to cuparenic acid, m. p. 158–160°,  $[\alpha]_D +63^\circ$  (c 2.4).

The fraction b. p. approx. 161° (56 → 89 %) was a crystalline mixture of sesquiterpene alcohols. A sample of this mixture (3.0 g) was separated by chromatography on alumina (200 g); elution with benzene gave cedrol (1.8 g), m. p. and mixed m. p. 87–88°,  $[\alpha]_D +10^\circ$  (c 2.1); further elution with benzene-ether (9:1) gave widdrol (1.0 g), m. p. and mixed m. p. 97–98°,  $[\alpha]_D +105^\circ$  (c 2.9).

The acidic fraction (37 g) on standing deposited crystalline material (6 g) which was filtered off, washed with light petroleum and recrystallised from methanol giving pure "Widdringtonia acid II", m. p. and mixed m. p. 191–193°,  $[\alpha]_D -96^\circ$  (c 1.9), infrared spectrum identical with that of authentic material. A part (12 g) of the remaining oil on quick distillation gave a low-boiling forerun (1.9 g, 90–125°/4 mm), a higher-boiling main fraction (8.3 g, 145–170°/4 mm) and a residue (1.6 g, discarded). The forerun showed five distinct spots when chromatographed on dimethyl sulphoxide-impregnated paper<sup>18</sup>, but none of these compounds were obtained in a crystalline state when eluted from a preparative (60 mg) chromatogram and this fraction was not further investigated. The higher-boiling, main fraction crystallised on standing. After repeated crystallisation from light petroleum it gave pure hinokiic acid (1.5 g), m. p. and mixed m. p. 169–171°,  $[\alpha]_D -85^\circ$  (c 2.1) infrared spectrum identical with that of authentic material. The remaining material, a constant melting mixture (135–138°) was conveniently separated by preparative paper chromatography. A sheet of paper (23 × 50 cm, Whatman 3MM) was impregnated with a mixture of dimethyl sulphoxide and benzene (3:2), dried in an oven (60°, 3 min) and then placed between glass plates to protect it from atmospheric moisture while the acid mixture was being applied. A chromatogram of the acid mixture (70 mg) was run on this paper in light petroleum (b. p. 60–71°) for 7 h. Two bands, at 12 and 20 cm from the starting line, were detected when the chromatogram was sprayed with water. These bands were cut out, extracted with ether and the ether solutions obtained were washed with water, dried ( $\text{Na}_2\text{SO}_4$ ) and the ether was evaporated. The slow moving acid (20 mg) was identified as euparenic acid, m. p. and mixed m. p. 158–160°,  $[\alpha]_D +63^\circ$  (c 2.1), infrared absorption identical with that of authentic material. The fast moving acid was hinokiic acid which was identified in the same way.

No tropolones could be detected in the total acidic fraction, in the distilled acidic fractions or in the ether or light petroleum-insoluble fractions of the acetone extract when these were chromatographed according to the method of Wachtmeister and Wickberg<sup>19</sup>.

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## REFERENCES

1. Erdtman, H. *Fourth International Congress of Biochemistry*. Vol. II — *Biochemistry of Wood* (1958).
2. Zavarin, E. and Anderson, A. B. *J. Org. Chem.* **21** (1956) 332.
3. Erdtman, H. and Thomas, B. R. *Acta Chem. Scand.* **12** (1958) 267.
4. Erdtman, H. and Norin, T. *Acta Chem. Scand.* **13** (1959) 1124.
5. Simonsen, J. and Barton, D. H. R. *The Terpenes* (2nd Ed.) Vol. III, Cambridge 1951, p. 75.
6. Hirose, Y. and Nakatsuka, T. *Gakkaishi Mokuzai* **4** (1958) 26.
7. Kawamura, J. *Bull. Imp. Forestry Expt. Sta., Japan* **30** (1930) 59, from Aikawa, T. and Tanaka, J. *Quart. J. Osaka Techn. Expt. Sta.* **8** (1958) 197.
8. Erdtman, H. and Pelchowicz, Z. *Ber.* **89** (1956) 341.
9. Nozoe, T. and Kafuku, K. *Bull. Chem. Soc. Japan* **6** (1931) 111.
10. Katsura *J. Chem. Soc. Japan* **63** (1942) 1483, from *Chem. Abstracts* **41** (1947) 3449.
11. Simonsen, J. and Barton, D. H. R. *The Terpenes* (2nd Ed.) Vol. III, Cambridge 1951, p. 113.
12. Kawamura, J. *Bull. Govt. Forestry Expt. Sta., Japan* **30** (1930) 59, from Akiyoshi, S. and Nagahama, S. *Bull. Chem. Soc. Japan* **30** (1957) 886.
13. Enzell, C. and Erdtman, H. *Tetrahedron* **4** (1958) 361.
14. Runeberg, J. *To be published shortly in Acta Chem. Scand.*
15. Lindberg, B. and Wickberg, B. *Arkiv Kemi* **13** (1959) 447.
16. Bluman, A. and Schulz, L. *Ber.* **64** (1931) 1540.
17. Treibs, W. *Ber.* **70** (1937) 2060.
18. Wickberg, B. *Acta Chem. Scand.* **14** (1960) *In the press*.
19. Wachtmeister, C.A. and Wickberg, B. *Acta Chem. Scand.* **12** (1958) 1335.

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