

The Chemistry of the Natural Order *Cupressales* \*VIII. Heartwood Constituents of *Chamaecyparis nootkatensis* —  
Carvacrol, Nootkatin, and Chamic Acid

BLENDA CARLSSON, H. ERDTMAN, A. FRANK, and W. E. HARVEY\*\*

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

Extensive studies on the extractable heartwood constituents of the natural order *Pinales*, with particular reference to the genus *Pinus*, which have been carried out in recent years in this laboratory<sup>1</sup> have yielded information of considerable interest, not only from the purely chemical view-point, but also in connection with the biological relationship between different species and genera. There seems every indication that similar investigations on other plant orders will be equally profitable, and the order *Cupressales* has been chosen for reasons which should perhaps be briefly outlined.

The order *Cupressales* is divided into two families, the rather heterogeneous *Taxodiaceae* which has only 15 species, and the more uniform *Cupressaceae*, about 150 in number, divided into 11 genera many of which are monotypic or have only a few species. No systematic study of a large genus has been carried out, but as has been pointed out previously<sup>2,3</sup>, it is known that the heartwood constituents in the family *Cupressaceae* are frequently of terpenoid type, and certain compounds have been isolated from more than one genus. The genera *Chamaecyparis*, *Thuja*, and *Thujopsis* have all yielded compounds ( $\alpha$ -,  $\beta$ -, &  $\gamma$ -thujaplicins and thujic acid) which although not terpenes are of the  $C_{10}$  type and are obviously closely related to the terpenes. This fact is particularly interesting, and despite the fact that two *Chamaecyparis* species, *C. obtusa*

\* In view of our widening interest in the order *Cupressales*, the general title of this series of papers has been changed and the publications under the general title "Antibiotic Substances from the Heartwood of *Thuja plicata* (D. Don)" will be regarded as Pts. I—VII of this series. Part VII *Acta Chem. Scand.* 5 (1951) 995.

\*\* A/B Kabi Research Fellow, 1950—51.

Sieb. and Zucc. and *C. formosensis* Matsum. both of East Asian origin, have been investigated in some detail<sup>4</sup>, *C. nootkatensis* (Lamb) Spach occupies a somewhat isolated position in the genus and consequently was selected for our purposes.

*Chamaecyparis nootkatensis* (yellow cedar, Alaska cedar, yellow cypress) is a moderately large tree which grows on the Pacific Coast of Canada and elsewhere. The heartwood possesses a yellowish colour, is relatively light, hard, and close-grained, and, because of its resistance to decay and its insect repellent properties, is in considerable demand in Canada for use in furniture manufacture. It is also used extensively for battery separators.

The study of the constituents of the heartwood of yellow cedar was commenced in this laboratory some time ago, but, as some of the original collaborators are no longer engaged in this work, and as a number of interesting and significant findings have been made, it appears desirable to give a brief survey of our results to date.

The only recorded investigation on the steam-volatile constituents of *C. nootkatensis* was carried out by Clark and Lucas<sup>5</sup> as far back as 1926. Although these workers isolated both the wood and leaf oils they confined their detailed study to the latter, identifying  $\alpha$ -pinene and limonene, and obtaining evidence, not entirely satisfactory, for the presence of  $\beta$ -pinene, sabinene and *p*-cymene.

We have limited our work to the heartwood components obtainable by steam distillation of the finely divided wood. The steam distillation proceeds in two fairly distinct stages: the initial product is a pale yellow oil which distills rapidly, but if the distillation is continued this oil is followed by further material which distills much more slowly and crystallises out in the form of colourless plates in the condensers and well-cooled distillate. This compound has been obtained pure and has been shown to be a hitherto unknown tropolone for which we have proposed the name *nootkatin*<sup>6</sup>. It is rather surprising that Clark and Lucas do not appear to have noticed the presence of any solid material in the crude distillate but it is possible that they did not continue the distillation sufficiently long or, more likely, that they used metal equipment. They record that the wood oil they obtained was deep red in colour, and this is probably a significant observation as we have noticed that the oil becomes deep red-brown during the course of working up. This is almost certainly due to the formation of small amounts of the ferric salt of nootkatin (*cf.* the ferric salt of tropolone<sup>7-9</sup>). Further, if iron or copper apparatus is used for the distillation, the yield of nootkatin is very small or nil, and in some cases we observed the formation of the green crystalline copper complex of nootkatin in the apparatus. Nootkatin can also be obtained by extraction of the wood with

acetone or alcohol, but no nootkatin was removed by prolonged extraction with cold, dilute sodium hydroxide solution.

The ultra-violet <sup>6</sup> and infra-red <sup>10</sup> absorption spectra of nootkatin indicate that it is a tropolone, and the chemical evidence is in accord with this conclusion. Thus nootkatin forms metal complexes and a monomethyl ether, exhibits no ketonic properties, and, on complete hydrogenation, gives an  $\alpha$ -glycol. It has the formula  $C_{15}H_{20}O_2$ , and thus contains one double bond in addition to those present in the cycloheptatrienolone entity. As mentioned in previous communications <sup>6,10</sup> evidence has been obtained that the extra-nuclear double bond is not conjugated with the ring system, and that it forms part of an isopropylidene structure. Recent work, details of which will be published shortly, has indicated that nootkatin contains two side-chains, one isopropyl and the other 3-methylbut-2-enyl, and it thus bears the same relationship to the sesquiterpenes as  $\alpha$ -<sup>11-13</sup>,  $\beta$ -<sup>12,14,15</sup> and  $\gamma$ -thujaplicins <sup>2,12</sup> do to the monoterpenes.

Nootkatin is obtained during the normal working up procedure in the phenolic fraction of the oil, and from the same fraction we have isolated significant amounts of carvacrol. This is of interest since, although carvacrol has been obtained from a number of other plant sources, it appears to be a rare constituent of the heartwoods of conifers, the only recorded instance of its isolation being from the wood of *Tetraclinis articulata* <sup>16</sup>.

The neutral components of the wood oil have not been investigated in detail as yet, but a preliminary study of the acidic fraction has resulted in the isolation of a new liquid acid for which we propose the name *chamic acid*, and indicated the presence of at least one other acidic substance. Chamic acid,  $C_{10}H_{14}O_2$ , possesses an unusually high optical rotation ( $+257.5^\circ$  in MeOH) and probably contains two rings and one double bond. Studies on its structure are proceeding and will be reported at a later date, but it is interesting to note the occurrence of an acid containing a seven-membered ring, thujic acid <sup>17</sup>, with the thujaplicins in *Thuja plicata* (western red cedar), and this fact invites the speculation that chamic acid and thujic acid may be related.

As mentioned above, the heartwood of *C. nootkatensis* possesses considerable resistance to insect attack and Clark and Lewis <sup>5</sup> stated that "the wood apparently contains in the oil some valuable preservative constituent". Docent E. Rennerfelt has tested nootkatin and chamic acid for fungicidal properties and has found that they are active. Even the neutral material which constitutes the main fraction of the steam-volatile products of the wood possesses some activity.

## EXPERIMENTAL

*Extraction of heartwood constituents.* The wood which has been used for this investigation has, for the most part, come from a single tree grown near Vancouver, Canada. A smaller sample of wood from a tree grown near Stockholm was obtained and this was found to give a significantly greater yield of nootkatin, the only component which was investigated in detail.

a. *Steam distillation.* The wood was disintegrated in a Wiley mill and steam distilled in batches of ca. 2 kg using a chromium-plated vessel and glass condensers. It is essential that no copper or iron apparatus should be employed if it is desired to isolate nootkatin in good yield. The first portion of the distillate contained an appreciable quantity of oil, but this was later replaced by nootkatin which separated in crystalline form in the condensers and the cold distillate. The distillation was continued until the condensate was clear by which time 120–150 l had been collected. This was saturated with salt and extracted twice with ether and the ether extracts united and evaporated. The resulting dark red-brown oil (ca. 55 g, 2.75 %) was taken up in ether (ca. 1 l) and extracted with saturated sodium carbonate solution ( $3 \times \frac{1}{2}$  volume), then with *N* NaOH ( $6-10 \times \frac{1}{2}$  volume) and washed well with water until no more material was extracted ( $3-4 \times \frac{1}{2}$  volume). Acidification of the sodium bicarbonate solution with hydrochloric acid gave an oil which was recovered by ether extraction ( $2 \times$  equal volume). Acidification of the sodium hydroxide solution plus washings gave a mixture of oil and crystalline material which was also recovered by ether extraction. The yields of acidic, phenolic, and neutral material varied somewhat from batch to batch but were approximately 3.5 g, 11 g and 40 g (0.18, 0.55, 2.0 %) respectively.

b. *Acetone extraction.* Finely divided wood (3.7 kg) was continuously extracted (Soxhlet) with ether (3 l) until the extract was no longer coloured (48 hrs). The material obtained by evaporation of the ether (102.5 g, 2.8 %) has not been investigated. The extracted wood was air-dried and then extracted in the same way with acetone, until extraction was complete (48 hrs). The dark red acetone solution was concentrated to small volume, and the light brown "membrane substances" (6 g) precipitated by the addition of excess ether, removed by filtration, and washed well with ether. The dark red ethereal solution was extracted successively with saturated sodium bicarbonate solution ( $3 \times$  equal volume), *N* NaOH ( $3 \times$  equal volume), and water ( $2 \times$  equal volume), then dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated to give a crude neutral fraction which was distilled with steam. The distillate, which contained oil, was saturated with salt and extracted with ether ( $2 \times$ ) and the ether extracts combined, dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated, giving the neutral material as a red-brown oil (9.1 g, 0.25 %).

The acidic fraction (2 g) recovered by acidification (HCl) and ether extraction ( $2 \times$ ) of the sodium bicarbonate solution was steam distilled and worked up similarly, giving 0.55 g (0.015 %) of purified material. Steam distillation of the crude phenolic fraction obtained from the sodium hydroxide extracts and the aqueous washings gave a distillate which deposited crystalline material on cooling and which was worked up as before, giving a semi-solid dark brown product (2 g, 0.05 %) from which nootkatin (1 g, 0.03 %) was isolated by distillation *in vacuo*.

c. *Alcohol extraction.* A sample of Swedish-grown wood (3 kg) was extracted as above using alcohol as solvent in place of acetone, and omitting the preliminary extraction with ether. The extract was worked up as before, yielding 2.8 g (0.09 %) of nootkatin.

*Attempted extraction of nootkatin by means of sodium hydroxide.* Cold dilute aqueous sodium hydroxide (24 l, 1 N) was allowed to percolate through finely powdered wood (830 g, Swedish origin) the percolate being run into excess of dilute hydrochloric acid. The wood was then washed with water (9 l) and the total percolate and washings worked up in the usual way. No nootkatin could be recovered from the extract, but steam distillation of the extracted wood led to the isolation of nootkatin (0.9 g) in about the expected yield.

*Isolation of nootkatin.* The dark brown viscous phenolic fractions slowly deposited crystalline material which was collected by filtration, washed with a little ether and petroleum ether (b. p. 40–60°) and recrystallised several times from ligroin giving pure *nootkatin* as colourless plates, m. p. 95°,  $[\alpha]_D^{20} \pm 0$  ( $c = 5$  in  $\text{CHCl}_3$ ).

Light absorption (in *n*-heptane)<sup>6</sup>:

Maxima,	238,	315 *	324,	357,	373 * $m\mu$ .
log $\epsilon$ ,	4.49,	3.90,	3.94,	3.79,	3.64
$\text{C}_{15}\text{H}_{20}\text{O}_2$ (232.31) Calc.	C 77.55	H 8.68	C-Me 17.64,	Mol. Wt. 232.3	
Found	» 77.6	» 8.76	» » 10.93, 10.94	» » <sup>6</sup> 248.6	

Distillation of the phenolic mother liquors *in vacuo* gave a low-boiling fraction (mainly carvacrol), followed by nootkatin which crystallised on cooling and was purified by crystallisation from ligroin as before. A higher-boiling fraction which distilled as a yellowish oil has not yet been investigated.

*Nootkatin* is almost insoluble in water and cold ligroin, but readily soluble in dilute sodium hydroxide solution and nonhydrocarbon solvents. With a limited amount of alcoholic ferric chloride it gives an intense red colour — with an excess an intense green colour, which is visible even in very dilute solution and serves as a sensitive test (*cf* tropolone<sup>7,9</sup>).

The *copper complex of nootkatin* obtained by shaking a chloroform solution of nootkatin with aqueous cupric acetate and evaporating the resulting dark green chloroform solution, crystallised from chloroform-ether as pale green needles, m. p. 234–5° (decomp.). It is insoluble in water and ether and sparingly soluble in acetone and alcohol, but readily soluble in chloroform and benzene.

*Nootkatin methyl ether.* Nootkatin (0.1 g) was dissolved in ether (2 ml) and a solution of pure diazomethane (1.2 moles) in ether (3.5 ml) was added. Nitrogen was evolved slowly, and the mixture was set aside in the refrigerator overnight. Evaporation of the ether gave an oil which was distilled twice *in vacuo*, giving *nootkatin methyl ether* as a pale yellow oil,  $n_D^{20}$  1.5840, which gives no colouration with ferric chloride.

$\text{C}_{16}\text{H}_{22}\text{O}_2$ (346.34) Calc.	$\text{OCH}_3$	12.6
Found	»	11.9

This product is probably a mixture of isomers.

*Decahydronootkatin.* Nootkatin (1.0 g) in glacial acetic acid (25 ml) was hydrogenated at room temperature and pressure in the presence of Adams' catalyst. Four moles of hydrogen were absorbed rapidly; thereafter the rate of absorption decreased markedly. The uptake of hydrogen ceased when a total of five moles had been absorbed. The catalyst was filtered off and the solvent removed *in vacuo*. The resulting oil was dissolved in ether (75 ml), and the ethereal solution was washed with 2N NaOH (2 × 10 ml) and water (2 × 10 ml), dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated. The resultant oil was distilled *in*

\* Point of inflection.

*vacuo*, giving *decahydronootkatin* as a colourless oil,  $n_D^{20}$  1.4820, which could not be induced to solidify, and probably consists of a mixture of isomers.

$C_{15}H_{30}O_2$ (242.39)	Calc.	C 74.32	H 12.48
	Found	» 75.64	» 11.95

*Isolation of carvacrol.* The phenolic mother liquor from which the crystalline *nootkatin* had been removed (9 g) was dissolved in chloroform (70 ml) and the solution shaken with an excess of aqueous cupric acetate. The resulting green chloroform solution was washed with water (3 × 50 ml) until the washings were colourless, dried ( $Na_2SO_4$ ), concentrated to small volume, and diluted with ether (100 ml). The green crystalline copper complex of *nootkatin* which separated slowly (0.85 g) was collected after one hour, and the ether solution was extracted with saturated sodium bicarbonate solution (2 × 25 ml), washed with water (2 × 25 ml) and extracted with 2N NaOH (3 × 25 ml) and then with water (2 × 25 ml). The sodium hydroxide and aqueous extracts were combined and acidified (HCl) and the resulting red-brown oil that separated was extracted with ether (3 × 25 ml). The ether extract was washed with water (2 × 10 ml), dried ( $Na_2SO_4$ ) and evaporated, and the resulting dark-brown oil (3 g) was distilled.

The first fraction (1 g) had b. p. ca. 115°/15 mm,  $n_D^{20}$  1.5289, and consisted mainly of *carvacrol* identified by conversion to the *phenylurethane*, m. p. 135–6° either alone or in admixture with an authentic sample (Analysis: Found C, 75.89; H, 6.97;  $C_{17}H_{19}O_2$  requires C, 75.81; H, 7.11 %) and by conversion to *2-methyl-5-isopropylphenoxyacetic acid* m. p. 149–50°, undepressed by an authentic specimen. The remainder of the material which distilled was a pale yellow oil b. p. ca. 170–80°/20 mm,  $n_D^{20}$  1.5340, which has not been further investigated.

*Isolation of chamic acid.* The viscous, brown coloured acidic fraction obtained from the steam distillation of the wood was taken up in acetone and an excess of cyclohexylamine in acetone was added. The white crystalline material which separated immediately was collected and recrystallised from acetone-methanol, giving the pure *cyclohexylamine salt of chamic acid*, m. p. 165–166°,  $[\alpha]_D^{20} + 164.5^\circ$  ( $c = 2.9$  in MeOH).

$C_{16}H_{27}O_2N$ (265.38)	Calc.	C 72.41	H 10.25	N 5.28
	Found	» 72.79	» 10.19	» 5.47

The cyclohexylamine salt is moderately soluble in cold water, and acetone, but readily soluble in ethanol and methanol.

Treatment of an aqueous suspension with dilute hydrochloric acid liberated an oil which was shaken out with ether (3 × ½ volume). The ether was washed with a little water, dried ( $Na_2SO_4$ ) and evaporated giving an oil which was distilled *in vacuo* giving pure *chamic acid* as a colourless oil, b. p. 142°/8 mm,  $n_D^{20}$  1.4998,  $D_D^{20}$  1.0632,  $[\alpha]_D^{20} + 257.5^\circ$  ( $c = 1.4$  in MeOH),  $[Ma]_D$  45.6.

$C_{10}H_{14}O_2$ (166.21)	Calc.	C 72.26	H 8.49	Equiv. 166	one C—Me 16.27
	Found	» 71.82	» 8.49	» 165	» » 6.68

The *p-bromophenacyl ester*, prepared in the usual way formed colourless leaflets from aqueous ethanol, and melted at 74.5–75°.

$C_{18}H_{19}O_3$ Br (363.75)	Calc.	C 59.51	H 5.27
	Found	» 59.53	» 5.47

The *S-benzylthiuronium salt*, crystallised from ethanol forming colourless plates, m. p. 153–4°.

$C_{18}H_{24}O_2N_2S$ (332.45)	Calc.	S 9.64
	Found	» 9.70

The mother liquors from which the cyclohexylamine salt of chamic acid had been isolated contain a further acid (or acids) which is being studied and will be reported on in a subsequent communication.

#### SUMMARY

A preliminary study of the steam-volatile constituents of the heartwood of *Chamaecyparis nootkatensis* has resulted in the isolation of carvacrol, the "sesquiterpene type" tropolone nootkatin,  $C_{15}H_{20}O_2$ , and a new acid,  $C_{10}H_{14}O_2$ , for which the name chamic acid is proposed.

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