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The Chemistry of the Natural Order Cupressales

42 *. Heartwood Constituents of *Tetraclinis articulata* (Vahl) Masters

YUAN-LANG CHOW and H. ERDTMAN

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

The heartwood of *Tetraclinis articulata* has been investigated. The following constituents have been identified: arabinose, carvacrol, thymohydroquinone, *p*-methoxythymol, thymoquinone, cedrol, hinokiol, hinokione, totarol and three new compounds, an *α,β*-unsaturated ketoalcohol $C_{16}H_{16}O_2$, the phenolic diterpene ketone totarolone $C_{20}H_{28}O_2$ and a diterpene hydroxy acid $C_{20}H_{30}O_3$. The wood also contains an *α,β*-unsaturated ketone, totarolenone, which is hydrogenated to totarolone. Evidence is presented for the presence of *β*- and *γ*-thujaplicin, cedrene and a sesquiterpene alcohol $C_{15}H_{24}O$.

Tetraclinis articulata (Vahl) Masters is a conifer growing in southern Spain and North Africa. It was formerly included in the northern hemispheric genus *Thuja* (*Th. articulata* Vahl) as well as in the southern hemispheric *Callitris* (*C. quadrivalvis* Ventenet).

Tetraclinis articulata is one of the few conifers possessing a lignin containing large amounts of syringyl groups. It is a somewhat isolated genus known from the tertiary period. The genus *Tetraclinis* is monotypic and for that reason it is, at the present stage, less suitable for chemotaxonomic purposes than large genera such as *e.g.*, *Pinus* and *Juniperus*. However, we have included it in our studies because we thought that it might be of some interest to compare its heartwood chemistry with that of other genera of the family Cupressaceae.

Long ago Grimal [1] subjected the steam volatile oil obtained from the fragrant, resistent wood to a brief investigation and isolated carvacrol, thymohydroquinone, thymoquinone and a neutral levorotatory oil.

In this preliminary examination we extracted with acetone the milled heartwood from a tree grown in Morocco. The extract was divided into ether-soluble and ether-insoluble products (I and II, respectively). From II a little arabinose was obtained by extraction with water. Fraction I was further divided into products soluble (III) or insoluble (IV) in light petroleum. From the latter thymohydroquinone was isolated. Fraction III was subjected to steam

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distillation and a volatile (V) and a non-volatile (VI) fraction were obtained. The volatile material was shaken successively with sodium bicarbonate and sodium hydroxide solutions yielding a very small, strongly acid fraction (not investigated) and a weakly acid fraction from which carvacrol and *p*-methoxythymol were isolated. The presence of *β*- and *γ*-thujaplicin was demonstrated by paper chromatography.

The neutral fraction (from V) contained thymoquinone and cedrol as well as a liquid which appeared to be a complex mixture of sesquiterpenes similar to many of the "natural cedrene" fractions described by various authors [2]. A gas chromatographic investigation showed the presence of one major and several minor components, none of which, apparently, corresponded to longifolene, thujopsene or cuparene. A small amount of a crystalline substance $C_{10}H_{16}O_2$, m.p. 70°, was also obtained from this fraction. It appears to be an *α,β*-unsaturated ketone containing a hydroxyl group and possibly a monosubstituted vinyl group. There was also a sesquiterpene alcohol which owing to its similarity to cedrol could not be isolated in a pure form, but which furnished a crystalline 3,5-dinitrobenzoate. The composition of this derivative indicated that the sesquiterpene might have the composition $C_{15}H_{24}O$.

The non-steam-volatile fraction VI was similarly divided into strong acids, weak acids and neutral compounds. From the weak acid fraction a very small amount of a diterpene acid $C_{20}H_{30}O_3$, m.p. 260—263°, was obtained. The infrared and ultraviolet spectra indicated that the compound contained a hydroxyl and a carboxyl group and at least one double bond (possibly an unsymmetrically disubstituted vinyl group). Acetylation gave what appeared to be a lactone acetate and an acid acetate. The amount isolated was too small for further investigation.

By a combination of distillation and chromatography, a surprisingly large amount of totarol [3] was isolated from the large neutral fraction (from VI). There were also small amounts of hinokiol and hinokione as well as a mixture of ketonic compounds which has not yet been separated into its components. According to its optical properties and hydrogenation experiments this ketone mixture appeared to consist of two closely related ketophenols, one being a simple ketone and the other an *α,β*-unsaturated ketone. They were called totarolone and totarolenone, respectively. The ketonic diterpenoids were also obtained from the light petroleum soluble fraction III by distillation and extraction of the high boiling fraction with a Girard reagent.

When the totarolone-totarolenone mixture was hydrogenated under mild conditions pure totarolone was obtained in almost quantitative yield.

The structures of hinokiol and hinokione as well as of totarolone and totarolenone have been discussed in earlier preliminary communications [4,5].

No compounds characteristic of *Callitris* have been obtained from *Tetraclinis* which shares with *Thuja* some tropolones. *Thuja*, however, appears to be characterised by sesquiterpenes belonging to the "*trans*-farnesyl group". *Tetraclinis* contains cedrol (*cis*-farnesyl type) like *Platycladus* (= *Biota*), *Chamaecyparis*, *Juniperus* and several other genera of the order Cupressales. Thymohydroquinone occurs in *Tetraclinis* as well as in *Juniperus*. *p*-Methoxythymol has been found in *Calocedrus* (Syn. *Heyderia* and *Libocedrus*), and carvacrol in several tropoloniferous genera. All these characteristics [6] indicate

affinities between *Tetraclinis* and the northern genera of the family Cupressaceae.

From a chemical point of view the co-occurrence of normal and rearranged diterpene phenols such as hinokiol and totarol is of particular interest and certainly indicates biosynthetic interrelations. Such interrelations have already been suggested by Wenkert *et al.*[7]

EXPERIMENTAL

Extraction. Milled wood (8 kg) was extracted continuously with acetone for 24 h. Half of the extract was used for preliminary studies. The acetone was evaporated and the dark brown product poured very slowly into ether (7 l) under vigorous stirring to give an ether soluble (I) and an insoluble (II) product. The precipitate (II) was ground with ether (returned to I) and then with water (insoluble material 64 g). The aqueous extract was treated with charcoal and evaporated to dryness and the residue crystallised from methanol-ethanol (1:1) giving arabinose (0.1 g).

The ether solution (I) was evaporated, yielding a tar (260 g). This was stirred vigorously with light petroleum (5 l). Soluble: (III), insoluble (120 g): (IV). Fraction (III) was evaporated and steam-distilled to give a volatile (V) and a non-volatile fraction (VI). The volatile products were mixed with light petroleum and extracted successively with 5 % sodium bicarbonate and 2 N potassium hydroxide solutions, giving a strongly acid fraction (traces), a weakly acid fraction (VII) (3.5 g) and a neutral fraction (VIII) (68 g). The non-volatile fraction (VI) was dissolved in ether and similarly divided into strongly acid (1.5 g), weakly acid (IX) (13 g) and neutral products (X) (157 g).

Alternatively the solution III was directly divided into neutral products, weak acids and strong acids.

Fraction VII was examined by paper chromatography using dimethyl sulphoxide impregnated paper. Spots were observed corresponding to carvacrol, β-thujaplicin, γ-thujaplicin (trace) and *p*-methoxythymol (or *p*-methoxycarvacrol). The presence of tropolones was also demonstrated by the formation of chloroform-soluble copper complexes.

Fraction VII (545 mg) was dissolved in light petroleum (10 ml), absorbed on silica gel (30 g) and eluted with benzene. The first eluate (60 ml) gave 40 mg, and the second (110 ml) 352 mg, of oil. The latter was converted into a mixture of phenoxyacetic acids which was fractionally crystallised to give carvacroxyacetic acid, m.p. and mixed m.p. 153–155°, and *p*-methoxythymoxyacetic acid, m.p. and mixed m.p. 135–137°. The I.R. spectra were identical with those of authentic specimens.

Part of the neutral fraction VIII (53 g) was distilled through a spinning band column. The following results were obtained (first figure = fraction number, second = boiling range at 10 mm, third = weight in g, fourth = n_D^{25}, fifth = $[\alpha]_D$):

1) 68–72°, 0.05, 1.4753, –; 2) 72–76°, 0.4, 1.4772, + 33°; 3) 76–82°, 0.2, 1.4819, –; 4) 82–86°, 6.2, –, –; 5) 86–91°, 0.55, 1.4982, –; 6) 91–94°, 0.8, –, –; 7) 94–101°, 1.1, 1.4972, –; 8) 101–106°, 0.4, 1.4975, –; 9) 106–110°, 2.4, 1.4979, –; 10) 110°, 19.7, 1.4982, –; 11) 110–120°, 1.7, –, –48.1°; 12) 120–122°, 0.7, –, –; 13) 122°, 4.2, –, –; 14) 122–127°, 0.3, –, –8.9°; 15) 127°, 2.3, –, –19.2°; 16) 127–129°, 1.0, –, –40.6°; 17) 129–142°, 0.6, –, –6.5°; 18) 142–145°, 0.35, –, –; 19) 145°, 4.1, –, –2.9°; 20) 145°, 2.05, –, –;

Fraction 4 solidified on cooling to yellow crystals which were recrystallised from light petroleum, m.p. and mixed m.p. with thymoquinone 46–47°.

Fraction 6 partially crystallised on cooling. The product was recrystallised from light petroleum to give colourless needles, m.p. 70° (unchanged after sublimation), $[\alpha]_D$ + 78°, ν_{max} 900, 990, 1060, 1685 and 3450 cm^{-1}. λ_{max} 234 mμ (ε 8350). (Found: C 72.0; H 9.5. $C_{10}H_{16}O_2$ requires: C 71.4; H 9.6). The amount was insufficient for further investigation.

Fraction 10 was slightly yellowish and was dissolved in light petroleum (10 ml) and absorbed on a column of alumina. Elution with light petroleum gave a colourless oil (17.7 g) followed by thymoquinone (0.8 g). Redistillation of the oil gave a constant boiling main fraction (115°/12 mm), $[\alpha]_D$ –54.4° ($CHCl_3$). The infrared spectrum was similar to that of the neutral "cedrene" described by Naves [2]. The G.L.C.-examination

showed a main peak and several minor peaks, none of which corresponding to longifolene, thujopsene or cuparene.

The I.R. and U.V. spectra of fraction 13 indicated the presence of an aromatic hydrocarbon. It was re-chromatographed on alumina (200 g). The main fraction (3.5 g) was distilled and had b.p. 58–60/0.05 mm, $[\alpha]_D$ −16°, n_D^{25} 1.5068.

Fraction 19 partially crystallised on cooling. A sample (4 g) was dissolved in light petroleum (20 ml) and adsorbed on a column of basic alumina (220 g). The column was eluted successively with light petroleum, light petroleum-benzene and benzene. 32 subfractions were collected. Fraction 19 (1–3) contained a mixture of hydrocarbons, $[\alpha]_D$ −20°. Fraction 19 (10–21) gave cedrol which was recrystallised from acetonitrile $[\alpha]_D$ + 6.5°. M.p. and mixed m.p. with pure cedrol 88–89°.

Fraction 20 showed a strong hydroxyl band. After removal of primary alcohols with phthalic anhydride the residual oil was treated with 3,5-dinitrobenzoyl chloride in pyridine, giving a crystalline product which was recrystallised from ligroin, m.p. 152–153°. It was probably a sesquiterpene alcohol 3,5-dinitrobenzoate. (Found: C 64.0; H 6.8; N 6.6. $C_{22}H_{26}O_6N_2$ requires: C 63.8; H 6.3; N 6.8.)

Fraction IV. This fraction was dissolved in ether, extracted with 1 N potassium hydroxide solution and the aqueous phase was acidified. The acid product was treated with hot benzene and the solution obtained evaporated to a small volume. A mixture of an oil and a crystalline compound was obtained, and the latter was identified as hydrothymoquinone, m.p. and mixed m.p. 145–147°.

The weakly acid *fraction (IX)* was dissolved in light petroleum. A solid (110 mg) slowly separated. This was recrystallised from benzene, m.p. 230–240°. The product was sublimed along a temperature gradient and the main fraction (80 mg) was recrystallised from methanol, giving colourless crystals, m.p. 260–263°, $[\alpha]_D$ −9° (in EtOH), λ_{max} 208 mμ (ε 11 500). ν_{max} 906, 1000, 1635, 1665, 3440 and 1705 cm^{-1}. (Found: C 74.4; H 9.3. $C_{20}H_{30}O_3$ requires: C 75.4; H 9.5.) This compound (50 mg), fused sodium acetate (150 mg), and acetic anhydride (2 ml) were heated on a steam bath for 2 h. Addition of ice water gave a precipitate which was recrystallised from methanol, yielding a mixture of large crystals and a crystal powder. The large crystals were separated and recrystallised from methanol to give a lactone acetate, m.p. 213–215°. (ν_{max} 1000, 1245, 1630 (weak), 1730 and 1800 cm^{-1}). The crystal powder was recrystallised from ligroin three times giving white crystals (acid acetate), m.p. 210–213°. ν_{max} 1230, 1635, 1695, and 1730 cm^{-1}. (Found: C 73.6; H 8.8. $C_{22}H_{33}O_4$ requires: C 73.3; H 8.95.)

The "neutral fraction" (X) consisted of a dark pitch (90 g). It was dissolved in light petroleum (200 ml) and adsorbed on neutral alumina (1.6 kg). The column was successively eluted with light petroleum, benzene, ether, ethyl acetate, acetone, and methanol.

The light petroleum eluate (5 l) gave only 1 g of a resin which was not further investigated. The benzene eluate (7.5 l) gave 32.4 g of a resin which after several weeks deposited crystals, which were distilled in a vacuum (b.p. 130–135/0.2 mm) yielding a solid, m.p. 115–128°. Recrystallisation from light petroleum gave colourless crystals, m.p. 127–128°, not depressed on admixture with authentic totarol, $[\alpha]_D$ + 45° (EtOH). The I.R. spectra of the two samples were superimposable. The substance gave totarol acetate, m.p. and mixed m.p. 124–125°, $[\alpha]_D$ + 51.6° ($CHCl_3$).

Ether (2.5 l) eluted a resin (12.5 g) containing mainly totarol and a small amount of ketonic material. More ether (9.5 l) eluted 25 g of a resin which was triturated with the same amount of light petroleum, yielding a crystalline product (2 g). The analysis of this product showed that it consisted of approximately equal amounts of totarolone ($C_{20}H_{28}O_2$) and totarolenone ($C_{20}H_{26}O_2$), (ν_{max} 806, 815, 1585, 1652, 1680 and 3380 cm^{-1}). Catalytic hydrogenation in ethanol in the presence of a palladium-charcoal catalyst gave a product, totarolone, m.p. 188–189.5°, $[\alpha]_D$ + 101.5° (in EtOH). (ν_{max} 810, 1590, 1685 and 3380 cm^{-1}; λ_{max} 208 mμ (ε 37 700), 222 shoulder (8000), 281 (1900) and 286 (1900)). (Found: C 79.9; H 9.3. $C_{20}H_{28}O_2$ requires: C 80.0; H 9.4.) The acetate (acetic anhydride-pyridine), crystals from methanol, had m.p. 113–115°, $[\alpha]_D$ + 96.6° (EtOH); (ν_{max} 805, 1210, 1704 and 1755 cm^{-1}). (Found: C 77.7; H 8.8. $C_{22}H_{30}O_3$ requires: C 77.2; H 8.9.) The 2,4-dinitrophenylhydrazone, crystals from ethanol, had m.p. 192–194°. (Found: C 65.2; H 6.6; N 11.45. $C_{26}H_{32}N_4O_5$ requires: C 65.0; H 7.0; N 11.6.)

Elution with ethylacetate (3 l) gave a resin (23 g) which was dissolved in light petroleum and kept in an ice box. A crystalline solid (5 g) was deposited which was recrystalli-

sed three times from ethanol and six times from benzene, yielding hinokione (0.9 g), m.p. 191–192°, $[\alpha]_D$ + 111.9° (in EtOH); (ν_{max} 1620, 1695 and 3440 cm^{-1}); λ_{max} 208 mμ (ε 16 900), 219 (7500) and 284 (2400) in ethanol, and 242 (ε 7100), 306 (ε 4590) in 0.1 N sodium ethoxide. (Found: C 80.1; H 9.3. Calc. for $C_{20}H_{28}O_2$: C 80.0; H 9.4.) The acetate, (pyridine-acetic anhydride), m.p. and mixed m.p. with an authentic sample of hinokione acetate 120–122°. ν_{max} 1215, 1702 and 1759 cm^{-1}; λ_{max} 208 mμ (ε 12300), 269 (1200) and 277 (1300). (Found: C 77.1; H 8.8. $C_{22}H_{30}O_3$ requires: C 77.2; H 8.8). Hinokione 2,4-dinitrophenylhydrazone: crystals from ethanol, m.p. 234–236°.

The combined ethanol-benzene mother liquors were evaporated and the residue dissolved in benzene and adsorbed on silica gel (100 g). Elution with benzene furnished small amounts of totarol. Elution with benzene-ether (98:2) gave a totarolone-totarolenone mixture, further elution with benzene-ether (95:5), hinokione (1.1 g) and with benzene-ether (70:30), hinokiol (30 mg) which was recrystallised from benzene. M.p. and mixed m.p. with an authentic sample 240–242°. Superimposable I.R. spectra (peaks at 1030, 3310 and 3560 cm^{-1}).

A fraction (600 g) corresponding to the "neutral fraction" X obtained from a new batch of wood was distilled in a vacuum giving the following fractions.

1) B.p. 80–82°/0.5 mm, (100 g); 2) 88–100°/0.1 mm), (24 g); 3) 98–120°/1 mm, (71 g); 4) 120–128°/0.5 mm, (17 g); 5) 120–139°/0.2 mm, (32 g); 6) 142–170°/0.2 mm, (142 g); 7) 173–175°/0.2 mm, (86 g); 8) a residue (110 g).

Fraction 7 was treated with Girard P reagent (15 g) in ethanol (200 ml) containing acetic acid (30 ml) and a mixture (2.5 g) of carbonyl compounds was isolated. This was hydrogenated in ethanol over palladium on charcoal and the resulting product dissolved in benzene and chromatographed on silica gel using benzene-ether (98:2) as the eluting agent. Totarolone (1.5 g) was obtained. Further elution with benzene-ether (95:5) gave hinokione (1 g).

The non-ketonic products were added to fraction 6 and this mixture was dissolved in 300 ml of light petroleum. On cooling crystallisation took place. The crystals were recrystallised from light petroleum and ethanol, giving totarol (39 g). The mother liquor contained mainly totarol (as shown by the I.R. absorption).

Fractions 4 and 5 were combined and re-distilled. The main fraction, b.p. 90–94°/0.2 mm, was cedrol. The fraction, b.p. 94–105°, contained mainly sesquiterpene alcohols and a compound which according to its I.R. spectrum (ν_{max} 1645, 1685, 2700 and 3620 cm^{-1}) was probably an α,β-unsaturated aldehyde. However, no pure compounds have yet been obtained from this material by chromatography on alumina.

The residue from the distillation of fractions 4 and 5 was dissolved in light petroleum. On standing in a refrigerator, a crude mixture (35 g) of totarolone-totarolenone, hinokione and a trace of hinokiol separated. Part of this mixture (4.4 g) was sublimed in a high vacuum to give a slightly coloured product (4.0 g). It was dissolved in benzene and chromatographed on silica (150 g) using benzene-ether (2, 5, and 25 % ether) as eluents. A totarolone-totarolenone mixture (1.8 g), hinokione (1.5 g) and hinokiol (70 mg) was obtained.

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