

The Chemistry of the Natural Order Cupressales

XX.* Heartwood Constituents of the Genus *Widdringtonia*

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The heartwoods of the five recent South African *Widdringtonia* species have been subjected to a comparative chemical investigation. A series of new sesquiterpenoid compounds have been isolated including the hydrocarbon widdrene, the alcohol widdrol, two sesquiterpene alcohols containing two oxygen atoms and a series of acids one of which, widdrenic acid, has also been obtained as an oxidation product of widdrene. Cedrol was usually present in these conifers and eudesmol was isolated from two species.

Superficially the family *Cupressaceae* appears to be a fairly recent, homogeneous group of conifers. However, in contrast to other families it is spread all over the world and there is evidence in favour of the view that the northern and southern genera have long been geographically separated. The family is therefore certainly old — though probably not one of the very oldest — and a comparison of the northern and southern genera is consequently a matter of considerable interest.

Some taxonomists regard the southern genera including *Actinostrobus*, *Callitris*, *Diselma*, *Neocallitropsis* (*Callitropsis*), *Widdringtonia*, *Fitzroya*, *Pilgerodendron* and *Libocedrus sensu stricto* as forming a sub-family *Callitroideae*. Of these genera *Callitris* was the subject of the classical studies of Baker and Smith¹ and the monotypic *Neocallitropsis* was investigated by Simonsen². *Diselma*³, *Pilgerodendron*⁴ and *Austrocedrus*⁵ (part of *Libocedrus sensu lato*) which are also monotypic have recently been investigated in this laboratory.

Guaiol and (—)-dihydrogeranic acid (callitrol) were found to be characteristic for *Callitris*. Recent unpublished work (Erdtman and Jamieson) showed the presence of eudesmol in *Callitris Roei* (Endl.) F. Muell. and it is quite possible that small amounts of eudesmol occur in other *Callitris* species also. No tropolones were found in any of the *Callitris* species investigated here.

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Pilgerodendron contained sesquiterpenes of the cadinene type but no characteristic constituent was isolated from the heartwood samples of *Diselmia Archeri* available. *Austrocedrus* remains the only known tropolone-producing conifer from the southern hemisphere.

The northern monotypic genus *Tetraclinis* (North Africa, southern Spain) has been included in the southern *Callitroideae* but this does not appear entirely justifiable, either geographically, botanically or chemically. The heartwood chemistry indicates on the contrary some affinities with the northern genera.

Of the southern genera, apart from two included in *Libocedrus sensu lato*, only *Callitris* and the African *Widdringtonia* contain more than one or two species and the latter were therefore selected for a comparative study. No chemical relationship to *Tetraclinis* was discovered.

Widdringtonia is a South African genus with five recent species, *W. cupressoides* (L) Endl., *W. dracomontana* Stapf., *W. juniperoides* (L) Endl., *W. Schwarzii* (Marloth) Mast. and *W. Whytei* Rendle. The heartwoods of these species are fragrant and resistant to insects and Docent E. Rennerfelt (private communication) has found that the heartwoods of *W. Whytei* and *W. juniperoides*, the only economically important species, are very resistant to wood decaying fungi (*Coniophora*, *Lentinus*, *Poria*).

We have been able to investigate all five species. The ether soluble part of the acetone extract of the heartwood was an oil possessing a pleasant "cedar wood" odour. There was insufficient of the oil of *W. cupressoides* for a detailed examination but it appeared to resemble that from *W. dracomontana*. Eudesmol was isolated from both of these closely related species. The oils from the latter and from the remaining species were similar although eudesmol was not isolated from the other three. This does not necessarily mean that eudesmol is absent from these other species. The oils appeared to consist mainly of sesquiterpenes with some higher boiling substances. There were only traces of monoterpenes or compounds of similar boiling point range present.

The compounds isolated and a very approximate estimate of the amounts present in the various species are listed in Table 1. The figures are subject to the uncertainties of the estimation and to the wide variations possible in biological material.

The oils were separated into neutral and acid fractions and the former were fractionally distilled. The hydrocarbon fraction consisted of at least two sesquiterpenes of which the lower boiling, $C_{15}H_{24}$, $[\alpha]_D - 107^\circ$, was the principal constituent. This compound, called widdrene*, appears not to have been isolated before but has since been encountered in *Platyclusus (Biota) orientalis*⁵ and in *Chamaecyparis thyoides* (Enzell, unpublished). Titration with perphthalic acid and the I.R. and U.V. spectra indicated the presence of a trisubstituted double bond. Like cedrene, widdrene must therefore be tricyclic.

Oxidation of widdrene with selenium dioxide gave an aldehyde widdrenal, $C_{15}H_{22}O$, m.p. $75-76^\circ$, which is a suitable derivative for the characterisation of widdrene. The compound possessed a strong cedar wood odour espe-

* Added in proof: According to Y. Hirose and T. Nakatsuka [*J. Jap. Wood Res. Soc.* 4 (1958) 26], the 'thujopsene' of J. Kawamura [*Bull. Imp. For. Exp. Stn. Japan No.* 30 (1930) 59] from *Thujopsis dolabrata* is essentially identical with widdrene.

Table 1. Constituents of *Widdringtonia* heartwoods.

Species:	<i>Whytei</i>	<i>junipe- roides</i>	<i>Schwarzii</i>	<i>cupress- oides</i>	<i>draco- montana</i>
(As a percentage of the air dried wood)					
Total oil (ether soluble)					
acetone extract)	12	15	7.5	0.5	4
Neutral	8	11	5	0.25	3
Alkali soluble	4	4	2.5	0.25	1
(As a percentage of the total oil)					
Neutral	65	65	65	50	80
Low boiling (terpenes etc)	2	0	0		0
Widdrene (C ₁₅ H ₂₄)	10	30	30		25
Widdrene II	2	5	3		present
Widdrol (C ₁₅ H ₂₆ O)	10	10	5	0	3
Cedrol	15	5	5		5
Eudesmol				3	10
Alcohol (C ₁₅ H ₂₆ O ₂) m. p. 154°		1			
Diol (C ₁₅ H ₂₆ O ₂) m. p. 138°					6
Higher boiling neutral material	15	20	10		10
Acids	30	20	10		10
Widdrenic acid (C ₁₅ H ₂₂ O ₂)	3	3	2		present
Acid II (C ₁₅ H ₂₂ O ₂)	3	5	5		present
Acid III, m.p. 160°					
Acid IV, m.p. 140°					
(last two incompletely characterised)					

cially when in a slightly impure or an autoxidised state. The U.V. spectrum shows that it is an $\alpha\beta$ -unsaturated aldehyde. Further oxidation with either silver oxide or with alkaline hydrogen peroxide gave the corresponding $\alpha\beta$ -unsaturated carboxylic acid, widdrenic acid, C₁₅H₂₂O₂, m.p. 169°.

Ozonolysis of widdrene gave a keto acid C₁₅H₂₄O₃, m.p. 166—168°, which on oxidation with hypobromite furnished a dicarboxylic acid, widdrene dicarboxylic acid, which readily formed an anhydride. This acid was also obtained from widdrenal by oxidation with alkaline hydrogen peroxide. It was recovered unchanged when treated with hydrogen chloride in chloroform and this, together with the result of the ozonolysis, makes the possibility of the presence of a *cyclopropane* ring or an unreactive double bond unlikely.

These reactions indicate that widdrene like cedrene contains the system C—C=C(CH₃)—C in one of the rings. As in the case of cedrene, dehydrogenation experiments failed to give any characteristic products.

The second slightly higher boiling hydrocarbon provisionally called widdrene II was present only in small amount. It was dextrorotatory and appears to be an isomer of widdrene but it is uncertain whether it is entirely pure.

Further fractional distillation of the neutral oils gave a crude crystalline mixture of sesquiterpene alcohols which were most readily separated by chromatography on alumina. *Widdringtonia Whytei*, *W. juniperoides* and *W. Schwarzii* contained cedrol which is widespread in the Cupressales and a new sesquiterpene alcohol, widdrol. Eudesmol was obtained from *W. dracomontana* together with cedrol and widdrol, while eudesmol only was isolated from the small amount of wood of *W. cupressoides*. No eudesmol was obtained from the first three species although its presence cannot be excluded.

Widdrol melts at 98° and has the composition $C_{15}H_{26}O$, $[\alpha]_D + 105^\circ$. It could be crystallised only with difficulty but after careful chromatography it could be purified by sublimation along a temperature gradient. The I.R. and U.V. spectra and a perphthalic acid titration indicated the presence of a double bond. It was attacked by ozone and was readily dehydrated by acidic reagents to a mixture of hydrocarbons. Widdrol was acetylated with difficulty and is therefore probably a tertiary bicyclic alcohol. Selenium dehydrogenation gave an oil from which a small amount of 1,7-dimethyl-4-isopropyl-naphthalene was isolated as the picrate. Since only a small yield of this unusual hydrocarbon was obtained, widdrol may perhaps not contain a preformed naphthalene ring and could be a spirane of the type considered by Ruzicka to constitute intermediates in the biosynthesis of cedrene.

The alkali soluble fraction of the oils contained little or no tropolones or phenols except for *W. cupressoides* which contained some material showing a phenolic type of U.V. spectrum but was insufficient for further investigation. The other four species gave resinous or crystalline acid fractions that all appeared to contain similar mixtures of acids. Four of the acids could be isolated in a crystalline form by crystallisation or by distillation of their methyl esters. One of the acids was identical with widdrenic acid (see above). Acid II, m.p. 190°, like widdrenic acid was $\alpha\beta$ -unsaturated. The purity and composition of the acids III and IV are not yet absolutely certain since analyses gave variable results. Judging from the U.V. spectra, acid III appears to be aromatic. On reduction of the ester with $LiAlH_4$ a product was obtained which gave a typical benzenoid spectrum (λ_{max} 263 $m\mu$, ϵ 900) and the preparations so far available yielded terephthalic acid on oxidation with dilute nitric acid. It was not affected by ozone under normal conditions. Acid IV had a different U.V. spectrum and was readily oxidised by ozone.

This exploratory study indicates that the genus *Widdringtonia* constitutes a rather homogeneous but distinctly separated group of general Cupressales character sharing with some northern genera, widdrene, cedrol and widdrol (the latter has recently been found in *Chamaecyparis thyoides*) and with some southern genera, eudesmol. The isolation of large amounts of sesquiterpene acids from these woods serves to emphasise the necessity of employing the extractive technique in investigations for the purpose of taxonomic comparison.

The co-occurrence of cedrol and eudesmol in at least *W. dracomontana* is interesting in view of the biogenetic schemes suggested by Ruzicka according to which their biosynthesis proceeds along different routes.

EXPERIMENTAL

Rotations were measured in chloroform; U.V. spectra in 95 % ethanol except where otherwise specified. The benzene-alcohol-water azeotrope was usually employed to dry solutions during evaporation. All m.p.s were taken on a hot stage.

Isolation of the oils. The ground, air-dried heartwood of each species was extracted with acetone for two days and the extract concentrated to a small volume. The material was then poured into a large volume of ether. The ether insoluble material which in the case of *W. Whytei* was obtained in large amount was discarded. The ether solution was shaken with water to remove acetone and then with 10 % sodium hydroxide solution to remove the acid fraction which was recovered by extraction with ether after acidification with dilute hydrochloric acid. Removal of the ether gave viscous oils. The neutral fractions from *W. dracomontana* deposited a considerable amount of a white material which was insoluble in light petroleum. The oil was therefore diluted with light petroleum, allowed to stand and further amounts of the material which separated were filtered off. This material was combined with that obtained by chromatography (see below).

All the neutral oils were treated in largely the same way and the separations of the constituents will therefore not be described separately except where they are divergent.

Neutral fractions. These oils after quick preliminary distillation under reduced pressure (about 10 mm) were fractionally distilled through a spinning band column. The hydrocarbons that distilled first (b.p. 115–130°/10 mm) were carefully fractionated and gave, after a small forerun, two fractions only. The material that followed (b.p. 140–150°/10 mm) contained the sesquiterpene alcohols and was distilled as a single fraction until the distillate ceased to crystallise when cold. The next fraction remained a viscous oil on standing; it was readily eluted from alumina by light petroleum and appeared to be the principal component of the higher boiling material. This and the remaining resinous material of still higher boiling point has not yet been investigated.

There was insufficient oil available from *W. cupressoides* for distillation.

Widdrene. The first hydrocarbon fraction was an oil of constant boiling point, b.p. 118–120°/10 mm, $[\alpha]_D -107^\circ$, n_D^{22} 1.5042. (Found: C 87.91; H 11.80. $C_{15}H_{24}$ requires C 88.16; H 11.84). The U.V. absorption spectrum showed only non-conjugated double bond absorption in the 210 $m\mu$ region. Titration with perphthalic acid in ethanol solution was almost complete after 36 h with the consumption of 0.9 mole of perphthalic acid. No attempt was made to isolate widdrene from *W. cupressoides* but widdrene with the same physical constants was isolated from the other four species. Widdrene possesses an odour very similar to that of cedrene, perhaps slightly stronger. The odour increased on standing, probably due to autoxidation.

Widdrenal. Widdrene (1 g) and selenium dioxide (600 mg) were heated in alcohol (20 ml, 95 %) under reflux for 4 h. The reaction solution was cooled, filtered, evaporated to dryness and the product sublimed under reduced pressure on to a cold finger and recrystallised from light petroleum to give *widdrenal*, m. p. 75–76°, $[\alpha]_D -27^\circ$ (c, 1.8), λ_{max} 252 $m\mu$, ϵ 6 000. (Found: C 82.12; H 10.3. $C_{15}H_{22}O$ requires C 82.52; H 10.15).

Widdrenic acid. Widdrenal (100 mg) was added to an aqueous suspension of silver oxide and the mixture heated for one hour on the steam bath. The suspension was acidified and extracted with ether. The ether solution was taken to dryness and the product recrystallised from ethanol and sublimed to give *widdrenic acid* (30 mg), m. p. 169°, $[\alpha]_D -86^\circ$ (c, 1.8), λ_{max} 245 $m\mu$, ϵ 5 000 (*n*-heptane). (Found: C 76.77; H 9.19. $C_{15}H_{22}O_2$ requires C 76.88; H 9.47).

Ozonolysis of widdrene. Ozone was passed through a solution of widdrene (10 g) in a mixture of methanol and ethyl acetate (1:1, 100 ml) at -75° until the solution turned blue. The solution was shaken with zinc dust activated with acetic acid, filtered, washed with water and then evaporated to dryness. The product was crystallised from methanol (5 g) and sublimed *in vacuo* to give a *keto-acid*, m. p. 166–168°, $[\alpha]_D -129^\circ$ (c, 2.1). (Found: C 70.91; H 9.59. $C_{15}H_{24}O_3$ requires C 71.40; H 9.59). The *keto-acid* (200 mg) was treated with excess sodium hypobromite solution for 3 h. The solution was extracted with ether and then sulphur dioxide was bubbled through until the colour was discharged. It was then acidified and extracted with ether and this ether extract was washed with water and taken to dryness to give a resin which was crystallised from methanol to give *widdrene dicarboxylic acid* (130 mg), m. p. 215°, $[\alpha]_D -57^\circ$ (c, 2.1). (Found: C 66.06; H 8.72. $C_{14}H_{22}O_4$ requires C 66.10; H 8.73).

The *dimethyl ester* was prepared by methylation with diazomethane and was purified by sublimation under reduced pressure, m. p. 71–73°, $[\alpha]_D - 59^\circ$ (c, 2.0). (Found: C 68.38; H 9.11. $C_{16}H_{26}O_4$ requires C 68.13; H 9.25).

The acid (100 mg) was refluxed in acetic anhydride (5 ml) for one hour and the reaction mixture was then evaporated to dryness. The resultant resin was sublimed on to a cold finger and crystallised from light petroleum-chloroform to give *widdrene dicarboxylic acid anhydride*, m. p. 113°, $[\alpha]_D + 39^\circ$ (c, 2.9). (Found: C 70.86; H 8.42. $C_{14}H_{20}O_3$ requires C 71.15; H 8.53).

Widdrene dicarboxylic acid was also obtained by treating widdrenal (300 mg) in methanolic potassium hydroxide solution (20 ml, 5 %) with hydrogen peroxide (2 ml, 30 %) for 3 h at room temperature. The reaction mixture was diluted with water, extracted with ether, acidified and extracted again with ether. This last ether extract was washed with water, evaporated to dryness and the resultant resin crystallised from ethyl acetate, m. p. and mixed m. p. 213–214°.

Widdrene II. The hydrocarbon fraction boiling slightly higher than widdrene was redistilled to give a constant boiling fraction, b. p. 128°/10 mm, $[\alpha]_D + 50^\circ$ (c, 3.0), n_D^{20} 1.5160. (Found: C 87.75; H 11.47. $C_{18}H_{24}$ requires C 88.16; H 11.84). (See forthcoming publication by Enzell and Erdtman on the purification and structure of the main component of this material.)

The sesquiterpene alcohol fractions. After the hydrocarbons had been distilled off, further distillation of the oil gave a large fraction, b. p. 140–150°/10 mm which crystallised on cooling. This material was dissolved in light petroleum and adsorbed on alumina. It was then eluted with benzene. The first fractions gave cedrol which was crystallised from aqueous methanol and then sublimed, m. p. and mixed m. p. 87–89°, $[\alpha]_D + 10^\circ$ (c, 2.0). Further elution with benzene-ether (1:1) gave a crystalline material which was sublimed, crystallised from cold light petroleum and resublimed along a temperature gradient to give *widdrol*, m. p. 98°, $[\alpha]_D + 105^\circ$ (c, 2.3), $\epsilon_{210} m\mu$ 1500. (Found: C 80.98; H 11.71. $C_{15}H_{26}O$ requires C 81.03; H 11.78). It gave a coloration with tetranitromethane.

The alcohol fraction from *W. dracomontana* was treated slightly differently. After removal of the sesquiterpenes the whole of the remaining material was chromatographed on alumina. The cedrol was eluted with benzene and then further elution with benzene-ether (9:1) gave crude crystalline eudesmol which was rechromatographed, recrystallised twice from light petroleum and sublimed, m. p. and mixed m. p. 83–84°, $[\alpha]_D + 45^\circ$ (c, 1.9). The infrared spectrum of this material was identical with that of an authentic sample of the natural mixture of isomers.

Elution with benzene-ether (1:1) gave widdrol and final elution with benzene-methanol (9:1) gave a product which was crystallised from chloroform-light petroleum to give a white powder which was combined with the material already isolated directly from the crude neutral oil by crystallisation from light petroleum (see above). This was recrystallised several times from chloroform-light petroleum to give a *diol*, m. p. 137–138°, $[\alpha]_D - 24^\circ$. (Found: C 74.81; H 11.54; M (Rast) 239. $C_{15}H_{28}O_2$ requires C 74.93; H 11.75; M 240). An active hydrogen determination gave 0.81 %; two active hydrogens require 0.83 %.

The neutral fraction from *W. cupressoides* was too small for distillation (2.0 g from 700 g of wood) and was therefore chromatographed directly. The benzene-ether (9:10) eluate crystallised partially and on crystallisation from light petroleum and sublimation gave eudesmol, m. p. and mixed m. p. 80–82°, $[\alpha]_D + 45^\circ$.

A sample of the neutral oil from *W. juniperoides* was chromatographed directly after first removing the widdrenes by distillation. In addition to widdrol and cedrol this chromatogram, on elution with ether-methanol (9:1) and recrystallisation of the material in this fraction from light petroleum gave an *alcohol*, m. p. 154°, $[\alpha]_D - 9^\circ$. (Found: C 75.40; H 10.80. $C_{15}H_{28}O_2$ requires C 75.58; H 11.00). An active hydrogen determination gave 0.47 %; one active hydrogen requires 0.42 %. The compound was unattacked by ozone.

Dehydrogenation of widdrol. Widdrol (1 g) and selenium (1 g) were heated at 300° for 24 h in an atmosphere of carbon dioxide. The product was distilled to give an oil (400 mg) with an ultraviolet spectrum corresponding to somewhat less than 50 % of a naphthalenoid hydrocarbon. The picrate of the hydrocarbon was recrystallised five times from methanol to give a small amount of a picrate, m. p. 91–93°, mixed m. p. with the picrate of 1,7-dimethyl-4-isopropyl-naphthalene, undepressed.

The acid fractions. The sodium hydroxide soluble fractions of the oils were dissolved in a small volume of ether and allowed to stand at 0° for some time. The material that crystallised was separated by crystallisation from a mixture of ether and light petroleum and then from methanol to give *acid I*, m. p. 169–170°, $[\alpha]_D - 86^\circ$ (c, 1.9), mixed m. p. with widdrenic acid prepared from widdrene (see above) undepressed. The material slightly less soluble in ether gave on recrystallisation from ether-light petroleum and from ethyl acetate, the *acid II*, m. p. 190–191°, $[\alpha]_D - 76^\circ$ (c, 1.9), $\epsilon_{220} \text{ m}\mu$ 5 000 (*n*-heptane). (Found: C 76.49; H 9.61; equiv.wt. (titration) 234. $\text{C}_{15}\text{H}_{22}\text{O}_2$ requires C 76.88; H 9.47; equiv.wt. 224).

An attempt was made to separate the two acids by distillation of the methyl esters, prepared by esterification with diazomethane, through a spinning band column. The boiling point curve (85 % b.p. 157–162°/10 mm) showed no obvious separation; the middle fractions contained a mixture that on crystallisation from light petroleum gave *widdrenic acid methyl ester*, m. p. 109–110°, $[\alpha]_D - 99^\circ$ (c, 2.0). (Found: C 77.69; H 9.85. $\text{C}_{16}\text{H}_{24}\text{O}_2$ requires C 77.39; H 9.73). Saponification of this compound by heating in 10 % methanolic potassium hydroxide solution for 3 h under reflux gave widdrenic acid, m. p. 169–170°. Attempted isomerisation of this ester or of the acid II ester by either acid or alkaline treatment failed.

The end fractions from the distillation through the spinning band column were chromatographed on alumina. Light petroleum eluted an ester which on saponification and recrystallisation from light petroleum gave a crystalline acid material, *acid III*, m. p. 160–161°, $[\alpha]_D + 63^\circ$ (c, 2.4), $\lambda_{\text{max}} 240 \text{ m}\mu$, ϵ 12 000. Benzene-light petroleum eluted an ester which was saponified by refluxing in 10 % aqueous methanolic potassium hydroxide solution for 2 h. Recrystallisation from light petroleum gave an *acid IV*, m. p. 138–140°, $\lambda_{\text{max}} 302 \text{ m}\mu$, ϵ 6 000. The acid III, m. p. 160–161°, could also be obtained from a mixture of the two by ozonolysis. These two acid products gave varying analytical results on repeated analysis but were apparently C_{15} compounds with two oxygen atoms.

The acid III (100 mg) was heated with nitric acid (15 ml, 10 %) for 24 h at 170°. The crystalline material that separated from the reaction mixture on cooling was filtered off (20 mg) and methylated with diazomethane. The ester was recrystallised from methanol to give terephthalic acid dimethyl ester, m. p. and mixed m. p. 143–144°.

Acid III (10 mg) was esterified with diazomethane and the reaction mixture was taken to dryness, dissolved in dry ether and refluxed with LiAlH_4 for 2 h. The solution was cooled, ethyl acetate was added, then water and acid. This mixture was extracted with ether and taken to dryness. The product showed a U.V. maximum at 263 $\text{m}\mu$, ϵ 900.

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