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# Fungicidal Properties of some Constituents of the Heartwood of *Tetraclinis articulata* (Vahl) Masters

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The heart wood constituents of conifers belonging to different families show great and apparently characteristic chemical differences [1]. In the *Pinaceae* phenols are common. Examples are pinosylvin and compounds belonging to the flavone-flavanone group in the genus *Pinus*, conidendrin in the genera *Picea* and *Tsuga* and again a flavanone, taxifolin, in *Pseudotsuga*.

In the *Cupressaceae*, phenols appear to be less common although a few instances are known. The known heart wood constituents of this family generally belong to the terpene group. Some of these are open terpenoids related to geranic acid. Examples are dihydrogeranic acid (rhodinic or citronellic acid = 'callitrol') in several *Callitris* species, dehydrogeranic acid in *Neocallitropsis araucarioides* (Compton) Florin *(Callitropsis araucarioides* Compton). Cyclic terpenoids occur in *Libocedrus formosana* Florin (shonanic acid) and in *Thuja plicata* D. Don (the thujaplicins, which, however, do not obey the isoprene rule, but which, nevertheless, are undoubtedly related to the true terpenes). Another constituent of Thuja plicata is the 'dehydroperillic acid', the proposed structure of which has now been disproved [2].

In 1904 Grimal [3] investigated the volatile oil in a yield of two per cent obtained by steam distillation of the wood of *Callitris quadrivalvis* Ventenat *(Cupressaceae)*. He segregated the oil into a phenolic (about one per cent of the wood) and a neutral fraction. Acids appear to be absent. The phenolic fraction consisted of carvacrol (I) and hydrothymoquinone (II). The neutral fraction yielded thymoquinone (III). Hence, contrary to what is known about the chemistry of the genus *Callitris*, this species produces phenols. They differ, however, from those occurring in the *Pinaceae* by being elaborated on a terpenoid basis. *Callitris quadrivalvis* is also known in the older litterature under

the name *Thuja articulata* Vahl. (Theophrast's '*Thujon*'). Taxonomists now recognise that this conifer is the representative of a monotypic genus *Tetraclinis*. Its generic name is *Tetraclinis articulata* (Vahl) Masters. This species is peculiar in another chemical sense. Its lignin contains syringyl groupe just as the angiosperms [4]. Only a few conifers share this peculiarity of *Tetraclinis* namely *Podocarpus amarus* (Blume) and *Podocarpus pedunculata* (Bailey) (N. O. *Podocarpales*). The separation of the genera *Tetraclinis* and *Callitris* is entirely in harmony with existing chemical evidence. *Tetraclinis articulata* inhabits southern Spain and north western Africa. It is the source of the sandarac resin.

The occurence of thymoquinone in *Tetraclinis* is strange. Simple quinones are infrequent plant products. This quinone together with the corresponding hydroquinone has been isolated from the oil of *Monarda fistulosa (Labiatae)* [5]. This plant contains an oxydase which causes the dehydrogenetion of the phenol to the quinone. The latter, therefore, may be a secondary product.

The fungicidal properties of the substances isolated by Grimal from the wood of *Tetraclinis* have been investigated and in addition also those of thymol (IV), an isomeride of carvacrol. The two monophenols are fairly strong fungicides surpassing hydrothymoquinone in activity. Thymoquinone possesses increased toxicity. In view of the facile dehydrogeneration of the hydroquinone by oxidases or otherwise, it appears probable that in the wood of *Tetraclinis*, hydrothymoquinone functions as a potential antibiotic which is transformed into the more potent quinone when the wood due to damage is exposed to light and air. This will be investigated as soon as fresh samples of the wood are available.

## MICROBIOLOGICAL SECTION

### I. Activity of thymol and the constituents of the wood of *Tetraclinis articulata* against wood destroying fungi

Varying amounts of thymol, carvacrol, hydrothymoquinone or thymoquinone were added to test tubes charged with sterilized malt extract agar. The substrates vere inoculated with small pieces of the mycelium of different fungi. The growth of the mycelia was measured at suitable intervals. Four parallel runs were made with each fungus. Tables 1—4 show the results in per cent of the growth in control experiments containing no toxic substances.

*Table 1. Relative growth in presence of thymol.*

| Fungus | Per cent thymol | | | | |
|---|---|---|---|---|---|
| | 0.001 | 0.002 | 0.005 | 0.01 | 0.02 |
| *Coniophora puteana* | 74 | 66 | 47 | 16 | — |
| *Lentinus lepideus* | 55 | 45 | 18 | — | — |
| *Merulius lacrymans* | 40 | 38 | 27 | — | — |
| *Poria vaporaria* | 80 | 55 | — | — | — |

*Table 2. Relative growth in presence of carvacrol.*

| Fungus | Per cent carvacrol | | | |
|---|---|---|---|---|
| | 0.002 | 0.005 | 0.01 | 0.02 |
| *Coniophora puteana* | 80 | 43 | — | — |
| *Fomes marginatus* | 86 | 59 | 16 | — |
| *Lentinus lepideus* | 80 | 56 | 18 | — |
| *Merulius lacrymans* | 41 | 14 | — | — |
| *Poria vaporaria* | 58 | 26 | — | — |

*Table 3. Relative growth in presence of hydrothymoquinone.*

| Fungus | Per cent hydrothymoquinone | | | |
|---|---|---|---|---|
| | 0.001 | 0.002 | 0.05 | 0.1 |
| *Coniophora puteana* | 73 | 34 | — | — |
| *Fomes marginatus* | 35 | 29 | 6 | — |
| *Lentinus lepideus* | 32 | 11 | — | — |
| *Merulius lacrymans* | 2 | — | — | — |
| *Poria vaporaria* | 65 | 46 | 14 | — |

*Table 4. Relative growth in presence of thymoquinone.*

| Fungus | Per cent thymoquinone | | | |
|---|---|---|---|---|
| | 0.002 | 0.005 | 0.01 | 0.02 |
| *Coniophora puteana* | 78 | 77 | 64 | — |
| *Fomes marginatus* | 99 | 90 | 37 | 14 |
| *Lentinus lepideus* | 109 | 74 | 49 | — |
| *Merulius lacrymans* | 52 | 47 | 14 | — |
| *Poria vaporaria* | 80 | 74 | 45 | 16 |

From Tables 1 and 2 it is seen that thymol and carvacrol are moderately strong fungicides preventing growth of the different fungi at a concentration of about 0.01 %.

Hydrothymoquinone (Table 3) is less toxic. Concentrations of about 0.05—0.1 % are needed to prevent growth. The activity is rather similar to that of phenol when tested on the same organisms [6]. *Merulius lacrymans* is checked by lower concentrations of hydrothymoquinone than the other fungi. This conforms with the known sensitivity of this fungus to pinosylvin [7] and to the thujaplicins [6].

Thymoquinone appears to be less toxic than thymol and carvacrol but possesses greater inhibitory activity than its hydrogenation product.

## II. Activity against the germination of conidia of *Polyporus annosus*

*Polyporus annosus*, the common "root rot fungus", forms conidia which contribute to the spreading of this fungus. Unpublished observations show that certain natural fungicides, for example, pinosylvin inhibits the germination of these conidia at lower concentration (0.0025—0.005 % solution) than the growth of the mycelium (in 0.01—0.02 % solution). It was therefore of interest to investigate the influence of the *Tetraclinis* fungicides on the germination of the conidia of *Polyporus annosus*.

The experiments were carried out in 0.5 % malt extract solution and are summarised in Table 5.

*Table 5. Inhibition of the germination of conidia of Polyporus annosus.*

| Substance | Concentration in per cent | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0.1 | 0.05 | 0.025 | 0.01 | 0.005 | 0.0025 | 0.001 |
| Thymol | − | − | (+) | + | + | ++ | ++ |
| Carvacrol | − | − | + | + | ++ | ++ | ++ |
| Hydrothymoquinone | − | +[1]) | +++ | +++ | +++ | +++ | +++ |
| Thymoquinone | − | − | (+) | + | ++ | ++ | +++ |

[1]) Saturated solution

− = 0 % germination
(+) = < 5 %
\+ = 5—20 %
++ = 20—50 %
+++ = 50—60 % germination
(= germination in the controls)

No remarkable difference between the inhibition of the growth of mycelia and the germination of the conidia is apparent from these figures. The low activity of hydrothymoquinone is, however, apparent.

## III. Activity against blue stain fungi and organisms related to yeasts

Spore suspensions (1 ml containing $10^6$ spores) were introduced into small paper boxes followed by sterilised malt agar solutions to which various amounts of the test substances were added. After five days incubation the growth was observed. The results are summarised in Tables 6—7. (— = no growth, (+) = isolated colonies, + = poor growth, ++ = good growth. All controles ++.)

*Table 6. Growth of various lower fungi in the presence of thymol and carvacrol.*

| Fungus | Per cent thymol | | | | Per cent carvacrol | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.05 | 0.02 | 0.01 | 0.005 | 0.05 | 0.02 | 0.01 | 0.005 |
| *Cladosporium herbarum* | — | — | ++ | ++ | — | (+) | ++ | ++ |
| *Ophiostoma piceae* | — | — | ++ | ++ | — | (+) | ++ | ++ |
| » *pini* | — | — | ++ | ++ | — | (+) | + | ++ |
| *Phialophora fastigiata* | — | — | (+) | ++ | — | — | + | ++ |
| *Phoma lignicola* | — | — | + | ++ | — | (+) | + | ++ |
| *Pullularia pullulans* | — | — | + | ++ | — | — | ++ | ++ |
| *Penicillium janthogenum* | — | (+) | + | ++ | — | + | ++ | ++ |
| *Rhodotorula glutinis* | — | — | + | ++ | — | — | + | ++ |
| *Torulopsis pulcherrima* | — | — | + | ++ | — | (+) | + | ++ |

*Table 7. Growth of various lower fungi in the presence of hydrothymoquinone and thymoquinone.*

| Fungus | Per cent hydro-thymoquinone | | | Per cent thymo-quinone | | | |
|---|---|---|---|---|---|---|---|
| | 0.05 | 0.02 | 0.01 | 0.02 | 0.01 | 0.005 | 0.002 |
| *Cladosporium herbarum* | — | ++ | ++ | — | ++ | ++ | ++ |
| *Ophiostoma piceae* | — | ++ | ++ | — | — | ++ | ++ |
| » *pini* | — | + | ++ | — | — | + | ++ |
| *Phialophora fastigiata* | — | + | ++ | — | (+) | + | ++ |
| *Phoma lignicola* | — | + | ++ | — | + | ++ | ++ |
| *Pullularia pullulans* | — | + | ++ | — | — | — | ++ |
| *Penicillium janthogenum* | — | + | ++ | — | + | ++ | ++ |
| *Rhodotorula glutinis* | — | (+) | + | — | — | + | ++ |
| *Torulopsis pulcherrima* | — | (+) | ++ | — | — | + | ++ |

As seen from these tables the limits of inhibition are approximately for thymol 0.01—0.02 %, for carvacrol 0.02—0.05 %, for hydrothymoquinone 0.02—0.05 % and for thymoquinone 0.01—0.02 %. All these fungi are inhibited by approximately the same amount of the various substances. Hydrothymoquinone possesses the lowest toxicity. *Penicillium janthogenum* appears to be somewhat more resistent than the other fungi tested. It is a common observation that moulds such as *Penicillium* and *Trichoderma* species are relatively resistant to natural fungicides. Mc Gowan found that fungi belonging to these genera are far more resistant to viridine and gliotoxine than other fungi. All fungi, however, were inhibited by approximately the same amount of mercuric cloride [8].

The toxicity of thymol to bacteria has already been investigated by several workers[9, 10].

## SUMMARY

The toxicity against wood destroying fungi of thymol and three substances (carvacrol, hydrothymoquinone and thymoquinone) occurring in the wood of *Tetraclinis articulata* (Vahl) Masters, has been investigated. Hydrothymoquinone is approximately as active as phenol. Conversion into thymoquinone enhances the toxicity. The simple phenols, thymol and carvacrol, are more efficient fungicides than hydrothymoquinone.

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