

## Constituents of Pine Heartwood

### XXVI. \* A General Discussion

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The investigation of natural products has in the past been mainly concentrated on substances which are of interest either because of their colour or because they have potential technical, pharmacological or biochemical value. Taxonomic considerations have seldom guided the choice of the substances investigated. In recent years a considerable amount of research has been carried out on the biochemical and organic chemical aspects of genetics, and this work is a necessary condition for future developments in the chemistry of the evolution of species. At the present stage useful contributions in this field can be made by systematic studies of the chemistry of suitable genera of plants and animals. For this purpose genera should be chosen in which the number of species is neither too small nor too large, and it must also be decided which organs of the plant or animal and which of the substances found in them are of the greatest taxonomic significance, which is often difficult. Those organs which were evolved far back in geological time provide the best material for systematic studies, specialised organs of more recent evolution being of less value. The best "taxonomic tracer substances" should occur in organs of the former type and have little-specialised functions. Such substances ought to be essentially indifferent to external influences and hence "conservative".

The conifers constitute a group of plants which ought to be well suited for studies on chemical taxonomy. They are not too numerous (about 560 species), and they are comparatively well investigated from a taxonomic point of view. Some genera are of suitable size, as for instance *Pinus* (80—90 species). This genus is well distinguished from other conifers; botanists divide it into two subgenera (sections), *Haploxyton* (Soft or White Pines) and *Diploxyton*

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(Hard Pines). (Recently, the names *Strobus* and *Eupitys* have been proposed for these subgenera by Rehder<sup>1</sup>.) Both subgenera can be traced back to the early cretaceous period and have thus existed for about 125 million years<sup>2</sup>.

Different botanists have subdivided the genus *Pinus* into smaller units in different ways. Shaw, in his monograph of 1914, divided it into four subsections and thirteen groups<sup>3</sup>. More recent handbooks contain systems of subdivision which are somewhat different from that of Shaw<sup>1, 4, 5, 6</sup>. The main differences between these systems, however, belong to the subgenus *Diploxylon* and are of little interest for this investigation, as will be seen below. Shaw's system has been employed in Table 1, Part XXV<sup>7</sup>.

An attempt to classify the pines from a chemical standpoint has been made by Mirov. In a recent paper<sup>8</sup>, he has collected all information available about the composition of turpentine from different *Pinus* species. He also reviews previous investigations on some other genera. In many cases, pines which according to botanists are closely related also give turpentine of similar composition, but sometimes two very intimately related pines may yield turpentine of quite different composition, as for instance *P. ponderosa* and *P. Jeffreyi*. There does not seem to be any fundamental difference between the turpentine of pines from the two subgenera, *Haploxylon* and *Diploxylon*.

It was considered to be of great interest to compare the chemistry of pine heartwood constituents with either of the systems of classification based on botanical evidence. The investigations described in this series have dealt mainly with the phenolic constituents of the heartwood, but the water-soluble constituents (sugars and pinitol) have also been investigated to some extent. Other extractable heartwood products, such as the resin acids, have been left entirely outside the investigation, because they do not seem to be characteristic for the heartwood and, furthermore, they are more difficult to isolate in a pure state.

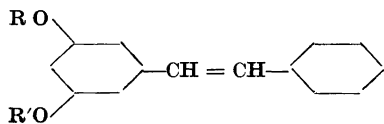
The non-digestibility of the heartwood of *P. sylvestris* in the normal sulphite process was investigated among others by Hägglund and his co-workers<sup>9</sup>. They arrived at the conclusion that it must be due to the occurrence of crystalline compounds in the heartwood. These compounds could be extracted from the wood with acetone, but not with ether, although they were soluble in ether when isolated. The acetone extract of pine heartwood contained ether insoluble compounds ("membrane substances"), later investigated by Erdtman<sup>10</sup>, which were supposed to protect the active compounds mechanically against ether extraction from the wood. From the acetone extract of *P. sylvestris*, Erdtman isolated two phenols, pinosylvin and its monomethyl ether (Part II<sup>11</sup>), which are responsible not only for the non-digestibility of pine heartwood but also for its high resistance to attack by wood-destroying

fungi<sup>12, 13</sup>. Further investigations revealed the presence of pinosylvin phenols in other pine species, and in addition hydroxylated flavones and flavanones were isolated. (Parts V—VII<sup>14</sup>.) These early investigations have now been continued and extended to include a large proportion of the recent pine species (about 25 %). (Parts IX—XVII<sup>15, 16</sup> and XIX<sup>17</sup>.) The general methods of extraction and fractionation used in these investigations are described in Part IX<sup>15</sup>. In one case, a new compound was isolated by these methods from a pine previously investigated (Part XVIII<sup>18</sup>).

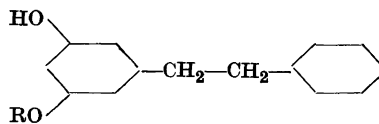
The introduction of paper chromatography for investigating heartwood extracts rendered a rapid and sensitive qualitative analysis of a large number of pines possible. (Part XX<sup>19</sup>.) A total of forty-eight species were investigated, including those which had been studied by the earlier methods (Part XXV<sup>7</sup>). Several new compounds were now discovered, and some of them subsequently isolated from the heartwood extracts and their structures elucidated. (Parts XXII<sup>20</sup>, XXIII<sup>21</sup>, and XXIV<sup>22</sup>.) Other substances of incompletely elucidated structure isolated during the earlier period of this series of investigations have been subjected to renewed studies. (Parts XXI<sup>23</sup> and XXIV<sup>22</sup>.)

#### BIOCHEMISTRY OF HEARTWOOD PHENOLS

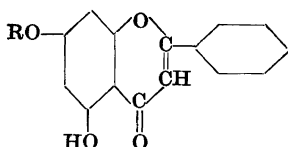
The following scheme gives the structures of all pine heartwood phenols at present known:



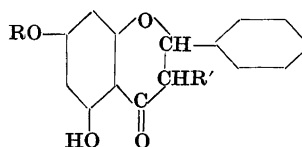
R = H, R' = H: Pinosylvin  
 R = H, R' = CH<sub>3</sub>: Pinosylvin mono-  
 methyl ether  
 R = CH<sub>3</sub>, R' = CH<sub>3</sub>: Pinosylvin  
 dimethyl ether



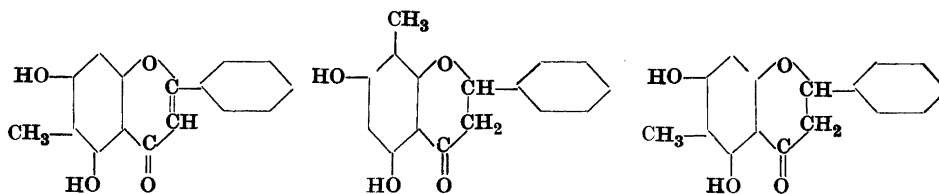
R = H: Dihydropinosylvin  
 R = CH<sub>3</sub>: Dihydropinosylvin  
 monomethyl ether



R = H: Chrysin  
 R = CH<sub>3</sub>: Tectochrysin



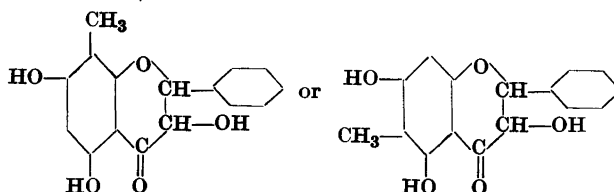
R = H, R' = H: Pinocembrin  
 R = CH<sub>3</sub>, R' = H: Pinostrobin  
 R = H, R' = OH: Pinobanksin  
 R = CH<sub>3</sub>, R' = OH: Pinobanksin  
 monomethyl ether



Strobochrysin?

Strobopinin?

Cryptostrobin?



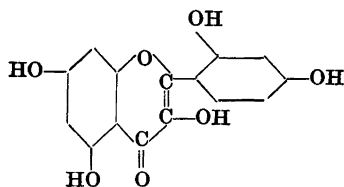
Strobobanksin

One of these fifteen substances, dihydropinosylvin, has not yet been isolated from any pine, but the paper chromatogram clearly shows that it is present in those pines which contain dihydropinosylvin monomethyl ether <sup>7</sup>. There are five pairs of compounds in which one (a flavanone or a dibenzyl) is the dihydro derivative of the other (a flavone or a stilbene). As will be seen below, this is of the greatest taxonomic importance. Three of the compounds listed above are 3-hydroxyflavanones (pinobanksin, its monomethyl ether and strobobanksin). The flavonols which can be formed by dehydrogenation of these substances have not so far been found in the heartwood of any pine. All the flavones or flavanones contain either a phloroglucinol or a methylphloroglucinol nucleus. The flavanones are all optically active.

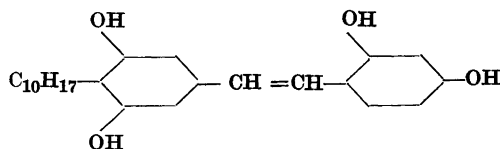
Only two of these compounds have been found in plants other than pines, namely chrysin and tectochrysin, which occur in poplar buds <sup>24</sup>.

All pine heartwood phenols isolated up to the present have one group in common, the unsubstituted phenyl group. Flavones and flavanones (C<sub>15</sub> compounds) are widely distributed in the vegetable kingdom, but only a few stilbenes (C<sub>14</sub> compounds) have been discovered so far. As far as the writer knows, flavones and stilbenes have never been isolated from one and the same plant except in the genus *Pinus*. In one case, however, such compounds have been found in two species belonging to the same genus. The wood of *Chlorophora tinctoria* Gaud., a South American tree, contains morin (3,5,7,2',4'-pentahydroxyflavone) <sup>25</sup>. Recently, a compound called chlorophorin has been isolated from *Chlorophora excelsa* Benth. and Hook f., an African tree with

highly durable wood. This compound has been shown to be a stilbene derivative <sup>26</sup>:



Morin



Chlorophorin

Like the unsubstituted phenyl group in pine heartwood phenols, the 2',4'-dihydroxyphenyl group is repeated both in morin and in chlorophorin. This group is rare in natural flavones, the 3',4' or 5'-positions generally being substituted. The occurrence of the same grouping in both types of compounds may possibly indicate some biochemical inter-relationship.

The occurrence of flavones and flavanones derived from methylphloroglucinol ( $C_{16}$  compounds) may be compared with the facile C-methylation of phloroglucinol. Perkin has reported C-methylation of quercetin by the action of methyl iodide in alkaline solution <sup>27</sup>. Thus, the  $C_{16}$  compounds may perhaps be regarded as the result of a biochemical methylation of a normal  $C_{15}$  compound.

As to the biological significance of the heartwood phenols, little is known except that the pinosylvin phenols serve as a defence against attacks from fungi, and possibly also from insects. The  $C_{15}$  compounds have no great fungicidal activity, and their insecticidal activity has not yet been tested. They may possibly serve as anti-oxidising agents.

#### HEARTWOOD PHENOLS AND BOTANICAL RELATIONSHIP

The distribution of the heartwood phenols in the genus *Pinus* is summarised in Tab. 1, Part XXV <sup>7</sup>. The great difference between pines from the two subgenera, *Haploxyton* and *Diploxyton*, is clearly demonstrated. Four substances, pinosylvin and its monomethyl ether, pinocembrin and pinobanksin are very common in both subgenera. Only two pines, *P. Lambertiana* and *P. peuce*, contain no pinosylvin phenols, but this may of course be due to individual variations. Pinocembrin is found in all pines investigated except perhaps *P. resinosa*. Pinobanksin is also widely distributed. The pines belonging to *Diploxyton* very seldom contain any phenols other than these four, the only exceptions being some of the unidentified compounds mentioned in Part XXV <sup>7</sup>. Pinostrobin and pinobanksin monomethyl ether were isolated from a

single (perhaps anomalous) specimen of *P. clausa* (Part XXII<sup>20</sup>) and are therefore not considered.

The subgenus *Haploxyton* contains four types of substances, which are not found in *Diploxyton*. The first two are flavones (chrysin, tectochrysin) and dibenzyls (dihydropinosylvin or its monomethyl ether). The latter were totally absent only in two species, *P. Lambertiana* and *P. peuce*, which also did not contain any pinosylvin phenols (see above). Thus, apparently the subgenus *Haploxyton* is able to dehydrogenate flavanones to flavones and to hydrogenate stilbenes to dibenzyls. Probably these two reactions are in some way biochemically connected. Since the stilbenes and the flavanones are distributed throughout the genus they are probably the primary products of biosynthesis.

The third group of heartwood constituents specific to *Haploxyton* consists of flavones and flavanones containing a methylphloroglucinol nucleus (strobopin, cryptostrobin, strobochrysin, strobobanksin, see Part XXIV<sup>22</sup>). These substances have only been found in five pines, *P. Lambertiana* and *P. monticola* from the Pacific coast of North America, *P. strobus* from North-eastern America, *P. parviflora* from Japan and *P. peuce* from the Balkan Mountains. All these pines belong to the group *Strobi* in Shaw's system<sup>3</sup>. (See Table 1, Part XXV<sup>7</sup>.) The same group is also contained in more recent systems<sup>1,5,6</sup>. Two other species from the same group, *P. Griffithii* (= *P. excelsa*) from the Himalayas and *P. ayacahuite* from Mexico, did not contain any C<sub>16</sub> compounds. Thus, the geographical distribution of the species seems to have no connection with the occurrence of C<sub>16</sub> compounds.

The fourth class of compounds specific to *Haploxyton* comprises flavones or flavanones methylated in the 7-position (tectochrysin, pinostrobin). With the exception of the anomalous specimen of *P. clausa* mentioned above, such compounds have not been found in *Diploxyton*.

*P. Lumholtzii*, a Mexican pine belonging to the subgenus *Diploxyton*, may constitute an interesting exception to the rules stated above, since it seems to contain dihydropinosylvin monomethyl ether and possibly also dihydropinosylvin. The other heartwood constituents are those ordinarily found in *Diploxyton*. (See Tab. 1, Part XXV<sup>7</sup>.) *P. Lumholtzii*, in Shaw's system<sup>3</sup>, belongs to the subsection *Parapinaster*, containing five species, which have some features in common with *Haploxyton*. In Pilger's system<sup>5</sup>, *P. Lumholtzii* belongs to the section *Pseudostrobus* containing among other species *P. Montuzemae*, *P. ponderosa* and *P. Jeffreyi*. If these dibenzyls actually did prove to be present in *P. Lumholtzii*, that species could be regarded as an interesting intermediate between *Haploxyton* and *Diploxyton*, but an investigation of many specimens of this somewhat inaccessible species is necessary to settle the point.

Of the unidentified compounds which have been found on the chromatograms, some give a colour reaction with the benzidine reagent<sup>19</sup>, whilst others can only be traced by their colour in ultraviolet light. The former compounds seem to occur more or less at random in a few species, but the fluorescent substances are more widely distributed. Compound D (Part XXV<sup>7</sup>) has been found in most pines from both subgenera, compounds E and F in *Diploxylon* and in a few pines from *Haploxylon*, but compound G only in *Diploxylon*. At present, nothing can be said about the chemical nature of these compounds, except that they must be weak acids, since they are extracted from the ether solution by sodium carbonate or hydroxide. Furthermore, the fluorescence of three of them (E, F, G) is increased by the addition of sodium carbonate.

#### WATER-SOLUBLE HEARTWOOD CONSTITUENTS

The occurrence of free *l*-arabinose in the acetone extract of pine heartwood<sup>10, 16, 17</sup> is remarkable, because apart from its discovery in the heartwood of another conifer, *Thuja plicata* D. Don<sup>28</sup>, this sugar does not appear to have been previously found in nature in the free state<sup>29</sup>. Of course, the possibility remains that the arabinose occurs in the form of a poly- or oligosaccharide, which is hydrolysed even under the very mild conditions of extraction (acetone at a temperature below 50°). Free glucose also seems to occur in the acetone extracts from most pines<sup>7</sup>.

Due to the difficulty of identifying pinitol on the chromatogram, it has not been possible to give an answer to the question whether this cyclitol is characteristic for the subgenus *Haploxylon* only. Considering however that pinitol has been isolated from six different *Haploxylon* pines but never from any *Diploxylon* pine, all evidence points in that direction.

The sugars and the fluorescent compounds D and E have also been found in sapwood (Part XXV<sup>7</sup>), in which their concentrations seem to be comparable with those in the heartwood. Pinitol is possibly a true heartwood substance. Erdtman was unable to isolate it from the sapwood of *P. strobus*<sup>14</sup>.

#### PHENOLS IN SAPWOOD AND BARK

As seen from Tab. 2, Part XXV<sup>7</sup>, traces of the most common heartwood phenols have also been found in the sapwood. This observation indicates that these compounds are generated in the cambium, from where they are transported centripetally through the sapwood via the medullary rays and finally accumulated in the heartwood. If the cambium is injured, the formation of heartwood phenols stops behind that injury<sup>13</sup>. The heartwood phenols are

apparently not distributed centrifugally into the bark. In the three species, the bark of which was investigated, not even traces of the heartwood phenols were detected. The bark is, however, rich in phenolic products of other kinds. From other conifers, an example is known of the same substance occurring both in the bark and the heartwood, namely the 3,5,7,3',4'-pentahydroxyflavanone in *Pseudotsuga taxifolia* Britt.<sup>30, 31</sup> Recently, a flavonol derivative of unknown structure has been isolated from the bark of *P. ponderosa*<sup>32</sup>. This compound, which is responsible for the yellow colour of the bark, is not identical with any of the heartwood phenols.

#### VARIATIONS WITHIN THE SAME SPECIES

Generally, only one wood sample from each species has been investigated, and this, of course, limits the possibility of drawing general conclusions. In the cases where two or more samples from one species have been investigated, however, the coincidence between the different samples was generally good as regards the number of substances found on the chromatogram. For instance, two samples of *P. Griffithii* (= *P. excelsa*), one from Himalaya and one from a pine cultivated in England, gave identical chromatograms. An exception was *P. clausa*, one sample of which contained pinostrobin and pinobanksin monomethyl ether, which were not found in other samples of the same species (Part XXII<sup>20</sup>). The percentage of each constituent in the heartwood may, however, vary within rather wide limits for different samples of the same species. Therefore, the yields of heartwood phenols reported from the preparative investigations<sup>15-17</sup> must not be regarded as average contents for the species investigated. An investigation of several hundred pines from different parts of Sweden (*P. sylvestris*) shows that the content of pinosylvin phenols in the heartwood varies between 0.4 % and 1.3 %, the average being 0.9 %. (Erdtman, Frank, and Lindstedt, to be published.)

#### MEMBRANE SUBSTANCES AND ETHER-EXTRACTABILITY OF THE PHENOLS

As mentioned earlier, the ether extract of *P. sylvestris* does not contain any appreciable amounts of phenols. The small quantities found have been ascribed to mechanical destruction of the "membranes" during the milling process<sup>9, 10</sup>. Some of the heartwood phenols are nearly always visible on the paper chromatogram of the ether extract, but the amount which is extracted with ether certainly varies very much for different species. In *P. Griffithii* (= *P. excelsa*) and *P. aristata*, a very large fraction of the total yield of heartwood phenols came from the ether extracts. (Parts XV and XVII<sup>16</sup>.) The



content of "membrane substances" in these pines was a little above 0.1 % of the heartwood, which is equal to the yield generally obtained from *P. sylvestris* (Part IV<sup>10</sup>). Thus, it seems as if the quantity of »membrane substances» in the heartwood, contrary to the assumption of Erdtman<sup>14</sup>, should have no great influence on the ether-extractability of the phenols, when different species are compared. However, as a general rule, the heartwood phenols can be extracted with ether to a higher extent in the subgenus *Haploxyton* than in the subgenus *Diploxyton*.

#### CONCLUSION

The present series of investigations shows that the heartwood phenols can be employed for a "chemical classification" of the genus *Pinus* and that there is a general agreement between botanical and chemical evidence. The two subgenera are clearly distinguished from each other. A further subdivision based on the heartwood phenols is possible only in a few cases. Considering that the investigation has included more than half of all recent *Pinus* species known, and that all groups except one are represented, it is hard to believe that any new phenolic constituents of fundamental significance will be found in the species which have not been investigated. "Trace compounds" may still remain to be discovered.

The systematic investigation of other chemical constituents of the pines, such as resin acids and their derivatives, would be an interesting complement to the present work. Another problem for the future would be to attempt hybridisation of different pines, especially between the two subgenera, and to investigate the heartwood phenols of the hybrids. The effect on the heartwood constituents of grafting a shoot of, for example, a *Haploxyton* pine on to a *Diploxyton* pine could also be studied.

#### SUMMARY

The investigation of the constituents of pine heartwood, as presented in previous parts of this series, has been reviewed and discussed. The following substances are characteristic for the two subgenera of the genus *Pinus*:

1) Subgenus *Haploxyton*: Stilbenes and dibenzyls; flavones and flavanones derived from phloroglucinol; and for the group *Strobi*, in addition, flavones and flavanones derived from methylphloroglucinol.

2) Subgenus *Diploxyton*: Stilbenes but not the corresponding dibenzyls; flavanones but no flavones.

The author is greatly indebted to Professor H. Erdtman, who entrusted to him the continuation of the studies in this field, for his interest in the work and for untiring help with the collection of wood samples. Skilful experimental assistance has been given by

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