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Flavanones from the Heart Wood of *Larix decidua* Mill. (*L. europea* D C.)

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The heart wood of conifers has proved to be a source of a wide variety of organic compounds [1]. Until recent times no investigation of the extractives of the heart wood of species belonging to the genus *Larix* appear to have been carried out. It was therefore deemed of interest to make such an investigation. The European larch, *Larix decidua*, has since long ago been cultivated in Finland, and was chosen as a representative of this genus.

By acidification of the sodium carbonate soluble part of the acetone extract of the heart wood, a crystalline precipitate was obtained which gave the colour reactions typical of 3-hydroxyflavanones [2]. It was readily soluble in hot water and crystallised therefrom upon cooling. It thus showed a striking resemblance to the dihydroquercetin (taxifolin) isolated by Pew [2] from the heart wood of *Pseudotsuga taxifolia*. By paper chromatography this precipitate was, however, shown to be a mixture of two compounds. Lindstedt [3] has recently shown the great value of paper chromatography for the identification of flavones and flavanones. In this case it was found more suitable to use another solvent mixture than those recommended by Lindstedt [3], *viz.* chloroform: alcohol: water (8 : 2 : 1, the bottom layer used). Use of this solvent gave after development of the chromatogram by dis-diazotised benzidine [4] two distinct spots. Different chromatograms gave somewhat varying R_F-values but there was always a definite difference between the two spots. The slower moving spot had R_F-values of about 0.2—0.4 and the faster moving 0.6—0.8. The slower moving spot was found to correspond exactly to the spot of taxifolin, when run simultaneously on the same paper strip. In the same way the faster moving spot was found to correspond to the dihydrokaempferol (3-hydroxynaringenin, coigue-flavanone) isolated by Pew [2] from the wood of *Nothofagus dombeyi*. (The author wishes to thank Dr. Pew and Prof. Erdtman for placing samples of taxifolin and coigue-flavanone to his

disposal.) For reasons given below the name aromadendrin will be given to this compound.

The conclusion that the two substances from *Larix decidua* should be identical with the two reference substances must however not be drawn from this evidence alone, because firstly both dihydroquercetin and dihydrokaempferol can exist in two diastereomeric forms which should not be expected to show any difference in R_F-values and secondly isomeric flavanones, with a different distribution of the hydroxyl groups should presumably not give very different R_F-values, and structural isomerism must not be ruled out.

In order to definitely prove or disprove the identity of the two pairs of compounds a preparative separation of the two substances in the mixture was carried out. This proved to be a rather difficult task. Fractional crystallisation failed to give any separation. Chromatography on alumina gave an eluate, which on a paper chromatogram showed only the presence of the faster moving compound, whereas the other was retained on the column and could not be eluted in a pure form. The use of silica-gel as absorbent gave no separation. Finally it was found that chromatography on a column of powdered cellulose, using chloroform: alcohol (9 : 1) as solvent gave a fairly good separation, giving only a small intermediate fraction containing both compounds.

The more easily eluted compound, as expected, proved to be the substance which on the paper chromatogram had the higher R_F-value. After purification it had m.p. 237—239° and corresponded to the formula $C_{15}H_{12}O_6$ and was for further characterisation converted into its tetra-acetate, m.p. 133—135°. This gave no depression of m.p. with the previously undescribed tetra-acetate of aromadendrin. Even the parent compounds gave no depression of m.p. on admixture, but this was found to be of rather little value for the purpose of identification, because even a mixture of aromadendrin and taxifolin produced only a very slight and difficultly observable depression.

The more difficultly eluted compound, which corresponded to the slower moving spot on the paper chromatogram had the composition $C_{15}H_{12}O_7$ and m.p. 238—240°. It was converted into its penta-acetate. This had m.p. 129—130°. The acetate of taxifolin prepared in the same way had m.p. 130—131° and no depression of m.p. was observed on admixture. On the other hand the acetates of aromadendrin and taxifolin whose m.p:s are rather close to one another show a depression of about 10° in the mixture test.

Graham and Kurth [5] have described the acetate of taxifolin as a non-crystallisable material of m.p. 82—85°. Obviously this was not pure.

It is thus evident that the two flavanones found in the heart wood of *Larix decidua* are identical with aromadendrin and taxifolin respectively. The two compounds were obtained from the chromatogram in the following yields:

aromadendrin 60 % and taxifolin 25 % of the original mixture. If the intermediate fraction containing both compounds is assumed to contain these in approximatively equal amounts, the proportion of aromadendrin to taxifolin is about 2 : 1. The total yield of the two flavanones together, based on air dried wood, is about 0.7 %.

When this work has almost completed there appeared a paper by Hasegawa and Shirato [6] describing the isolation from the heart wood of *Larix leptolepis* of a compound claimed to be identical with distylin. Distylin had earlier been isolated from the heart wood of *Distylium racemosum* [7] and shown to be a racemic dihydroquercetin [8].

Through the kindness of Dr. Hasegawa who sent a sample of his distylin, it was possible to submit it to a paper chromatographic analysis. This showed two spots corresponding exactly to the spots obtained from the mixture of flavanones from *Larix decidua*. Hasegawa's distylin is thus not a pure compound but presumably a mixture of aromadendrin and taxifolin. The amount available was, however, too small for a preparative separation and definite identification of these compounds.

In addition to the already mentioned occurrence of a dihydrokaempferol in *Nothofagus dombeyi* [2] there are a few other reports on the occurrence in nature of compounds with the same constitution. Uoda, Fukushima and Kondo [9] isolated from the wood of *Cercidiphyllum japonicum* a substance called katuranin (katsuranin), which was shown to be dihydrokaempferol. Later King and Acheson [10] isolated a substance with the same composition from the wood of *Afzelia* spp., which King [11] recently has shown to be identical with katuranin. Cohen and Hillis [12] have recently shown that aromadendrin, first isolated from the wood of *Eucalyptus* spp. by Maiden and Smith [13], is also a dihydrokaempferol. Dr. Cohen has been kind enough to provide the author with a generous sample of aromadendrin, from which a crystalline acetate easily could be obtained. This had m.p. 136—137° and gave no depression of m.p. with either the acetate of the dihydrokaempferol from *Nothofagus dombeyi* [2] or the acetate of the dihydrokaempferol from *Larix decidua*. The parent compounds can thus be considered identical and the name aromadendrin thus applies to all these three compounds.

Uoda, Fukushima and Kondo [9] report m.p. 128—129° for the acetate of katuranin. Acetylation of a sample of katuranin kindly provided by Dr. Hasegawa gave, however, an acetate with m.p. 134—135°. This gave no depression of m.p. when mixed with aromadendrin acetate. Even the optical rotation of katuranin is in agreement with the value reported by Cohen and Hillis [12] for aromadendrin. It is thus evident that even katuranin is identical with aromadendrin.

EXPERIMENTAL

Extraction of the heart wood. The finely powdered wood (5.5 kg) was extracted for 48 hours with acetone in a continuous extractor. Most of the acetone was distilled off from the dark coloured extract. The remaining viscous liquid was poured into ether. A small amount (4 g) of an amorphous precipitate was removed by filtration. The ether solution was then shaken with portions of sodium carbonate solution until no precipitate was obtained upon acidification. The combined sodium carbonate extracts were acidified with dilute sulphuric acid and the precipitate (94 g) removed by filtration. This contained still an appreciable amount of impurities, and was purified by dissolving in a small amount of ether and adding light petroleum in small portions. The addition of the first portions of light petroleum caused the precipitation of a resinous material. The later portions of light petroleum gave a crystalline, light yellow precipitate (37 g).

Separation of aromadendrin and taxifolin. The mixture (1 g) of the two flavanones, obtained as described above, was dissolved in chloroform: alcohol (9 : 1, 200 ml) and chromatographed on a column of powdered cellulose (37 × 1.8 cm). Elution was carried out with the same solvent mixture. The eluate was taken in fractions of about 200 ml each. Seven such fractions were obtained. Each fraction was then investigated by paper chromatography using chloroform: alcohol: water (8 : 2 : 1; bottom layer) as mobile phase. The chromatograms were developed by dipping the paper strip in a solution of bis-diazotised benzidine [4]. Because preliminary experiments with the two flavanones had shown that the R_F-values of the spots were rather variable, samples of authentic aromadendrin and taxifolin were always run simultaneously on the same paper strip. In this way the spots obtained from the different portions of the eluate could be definitely identified.

The fractions I—III showed the presence of only aromadendrin and fractions VI—VII of only taxifolin, whereas IV—V contained both compounds.

Aromadendrin. From the combined fractions I—III the solvent was removed *in vacuo* leaving a colourless crystalline residue (600 mg). This was purified by recrystallisation from chloroform-alcohol. M.p. 237—239°. $[\alpha]_D^{20}$ + 45° (c, 0.93 in equal volumes of acetone and water). Pew [2] records m.p. 237—241° and $[\alpha]_D^{20}$ + 45° in the same solvent mixture for aromadendrin from *Nothofagus dombeyi*. Aromadendrin from *Eucalyptus* spp. has m.p. 247—248° and $[\alpha]_D^{20}$ + 51.5° in the same solvent mixture [12]. Katuranin has $[\alpha]_D^{20}$ + 51° (c, 1.03 in the same solvent mixture), (measured on a sample provided by Dr. Hasegawa). A mixed m.p. determination showed no depression. ($C_{15}H_{12}O_6$ requires C, 62.5; H, 4.2; found C, 62.6; H, 4.4%).

The tetra-acetate was prepared by dissolving the flavanone in acetic anhydride and adding a drop of pyridine. After standing overnight the mixture was poured into water. The precipitate thus formed was recrystallised from methanol or ether. M.p. 133—135°. ($C_{23}H_{20}O_{10}$ requires C, 60.5; H, 4.4; found C, 60.3; H, 4.7%). When mixed with either the acetate of aromadendrin from *Nothofagus dombeyi* [2], m.p. 135—136°, or with the acetate of aromadendrin from *Eucalyptus* spp.[12], m.p. 136—137°, both acetates being prepared in the same way as described above, no depression of m.p. was observed.

Taxifolin. From the combined fractions VI and VII obtained in the chromatography the solvent was removed *in vacuo* yielding a slightly yellow crystalline residue (250 mg). This was recrystallised from chloroform containing a small amount of alcohol. M.p. 238—240°, $[\alpha]_D^{20}$ + 51° (c, 0.98 in equal volumes of acetone and water). Pew records m.p.

240—242° and $[\alpha]_D^{20}$ + 46 in the same solvent mixture for taxifolin from *Pseudotsuga taxifolia*. ($C_{15}H_{12}O_7$ requires C, 59.2; H, 4.0; found C, 58.4; H, 4.0%).

The penta-acetate was prepared in the same way as described above for the preparation of the acetate of aromadendrin. After recrystallisation from methanol it had m.p. 129—130°. ($C_{25}H_{22}O_{12}$ requires C, 58.3; H, 4.3; found C, 58.2; H, 4.3%). No depression of m.p. was observed when mixed with the acetate of taxifolin of m.p. 130—131°, prepared in the same way.

SUMMARY

Dihydrokaempferol and dihydroquercetin have been isolated from the heart wood of *Larix decidua*. Mill. The former has been shown to be identical with aromadendrin previously isolated from *Nothofagus dombeyi* and *Eucalyptus* spp. The latter has been shown to be identical with taxifolin previously isolated from *Pseudotsuga taxifolia*. The two flavanones were separated by chromatography on a column of powdered cellulose.

REFERENCES

1. Erdtman, H. in Cook, J. W. *Progress in Organic Chemistry*, London 1952, p. 22.
2. Pew, J. C. *J. Amer. Chem. Soc.* **70** (1948) 3031.
3. Lindstedt, G. *Acta Chem. Scand.* **4** (1950) 448.
4. Koch, J. E., and Krieg, W. *Chem. Ztg* **62** (1938) 140.
5. Graham, H. M., and Kurth, E. F. *Ind. Eng. Chem.* **41** (1949) 409.
6. Hasegawa, M., and Shirato, T. *J. Chem. Soc. Japan* **72** (1951) 279, *C.A.* **46** (1952) 3050.
7. Kondo, T. *J. Faculty Agr. Kyushu Univ.* **10** (1951) 79.
8. Kondo, T. *J. Faculty Agr. Kyushu Univ.* **10** (1951) 101.
9. Uoda, H., Fukushima, and Kondo, T. *J. Agr. Chem. Soc. Japan* **19** (1943) 467; *C. A.* **45** (1951) 9136.
10. King, F. E., and Acheson, R. M. *J. Chem. Soc.* **1950** 168.
11. King, F. E. *private communication.*
12. Cohen, W. E., and Hillis, W. E. *private communication.*
13. Maiden, J. H., and Smith, H. G. *Proc. Roy. Soc. N.S.W.* **29** (1895) 30.

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