Phenolics from *Larix* Needles XIV. Flavonoids and Phenolic Glucosides and Ester of *L. decidua* *

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Eleven flavonoids, four phenolic glucosides and one sugar ester were isolated from needles of *Larix decidua* and identified as: kaempferol, its 3-arabinoside and 3-rutinoside, quercetin-3-arabinoside, isorhamnetin-3-arabinoside and 3-(*p*-coumarylglucoside), laricitrin-3-glucoside and -3-rutinoside, myricetin-3-glucoside, syringetin-3-glucoside and apigenin-7-glucoside; the β-glucosides of *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid and ferulic acid, and the glucose ester of vanillic acid.

Introduction

Contrary to heartwood and bark, leaves of *Pinaceae* species have comparatively little been investigated for phenolic constituents [1]. Therefore, in our laboratory investigations on *Larix* leaf phenolics were started in 1969 [2]. *Larix decidua* leaves have previously been analysed for lipids [3], sterols [4], O-methylinositols [5], organic acids [6], main flavonoids [7] and for acylated flavonol glycosides [8]. In the present study some additional information is given on leaf phenolics which were mainly present in comparatively low concentration.

Results and Discussion

In the successive fractions obtained by the first polyamide column chromatography elution with water gave the esters and glycosides of the lower phenolics, of which the glucose ester of vanillic acid and the β-glucosides of *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid and of ferulic acid were identified. Alkaline hydrolysis and emulsine and acid hydrolysis showed the presence of both more phenolic esters and β-glucosides, but the amounts were too low for complete identification. *Ortho*-dihydroxy acids like for instance protocatechuic acid, were not detected.

The 40% methanol fraction mainly contained vitexin and its xyloside (published before ref. 7), and only traces of flavonol glycosides, possibly triosides, in too low a concentration for further purification.

The main amount of flavonol glycosides, both biosides and monosides, was found in the 60% methanol fraction, which was further separated on polyamide with CM 1. In addition to the major compounds kaempferol-, quercetin-, and isohamnetin-3-glucoside, and quercetin-, isorhamnetin- and syringetin-3-rutinoside (published before ref. 7) further purification by repeated band-chromatography yielded the 3-glucosides of myricetin, laricitrin (= 3′-methylmyricetin) and syringetin, the 3-arabinosides of kaempferol and isorhamnetin, and the 3-rutinosides of kaempferol and laricitrin. Free kaempferol was also found, but its occurence is probably due to some hydrolysis during the extraction procedure. No indication of it was found in preliminary screening experiments. New for larch was the appearance of the flavone apigenin, found in the form of its 7-glucoside. Trace amounts were also present of a 3-glucoside of a flavonol with all the properties of kaempferol, being however slightly more lipophilic and suggestive of a ring methylated kaempferol derivative.

For the flavonols the main pattern of glycosidation in all larch needles investigated (*cf.* [11]), found here for *L. decidua* as well, is 3-glucosidation, with a second occurrence of 3-rutinosides, whereas trace amounts occur of 3-arabinosides. One other diglycoside, however, a syringetin-3-diglycoside isolated in trace amount from the 60% methanol fraction, gave mainly xylose on acid hydrolysis.

Most acylated compounds were found in the 80% and 100% methanol fractions. In the 80% fraction these were mainly the ferulylglucosides of the four main flavonols published before [8] together with the coumaryl derivatives of kaempferol-3-glucoside and kaempferol-3-arabinoside and a *p*-coumaric acid ester of a flavanone glycoside, which was not completely identified. According to UV spectral data and hydrolysis products the most probable structure of the latter is naringenin-5?-(*p*-coumarylglucoside). In addition to the acyl derivatives some flavonol glycosides were present as well, among which myricetin-3-glucoside and low concentrations of quercetin-3-arabinoside were found.

Acylated flavonol glycosides were also found in the 100% methanol fraction, together with some

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free flavonols and some blue fluorescent phenolic acid derivatives which were not identified. The acyl derivatives in this fraction were *p*-coumarylglycosides of which two were identified as kaempferol- and isorhamnetin-3-(*p*-coumarylglucoside). For a third coumarylglycoside, a quercetin derivative, the concentration was too low for identification.

Synthesis of acyl derivatives of the main flavonol-3-glucosides with both ferulic and *p*-coumaric acid seems quite a general procedure in needles of *L. decidua*. Only for myricetin and laricitrin acylated derivatives were not found as yet. However, trace amounts may easily be missed in view of the rather tedious purification procedure. Also, for the common C-glycoside, vitexin, acylation occurs; one of the apigenin derivatives isolated during a previous extraction [7] indeed gave ferulic acid and vitexin on alkaline hydrolysis.

Again, the easy formation of acyl derivatives in this case, together with the fact that acylated flavones and flavonols have been found in species of such divers families as Tiliaceae (*Tilia* [12, 13]), Solanaceae (*Petunia* [14]), Leguminosae (*Pisum* [15]), Compositae (*Anaphalis* [16]), Betulaceae (*Corylus* [17]) and Gentianaceae (*Gentiana* [18]), and in almost all species investigated by our group, including Pinaceae (*Pseudolarix* [19], *Cedrus* [20, 21] and *Pinus* (to be published, also [22]), Asclepiadaceae *(Hoya)*, Crassulaceae *(Sedum)* and most probably Cucurbitaceae *(Cucumis)* and Labiatae *(Coleus)*, supports our earlier suggestion [19] that acylation is very common in the plant kingdom in spite of the scarce number of reports on this type of compounds.

Comparing *Larix decidua* leaves with those of other larch species, they appear comparatively rich in their array of acylated constituents Probably, however, this is mainly due to the very high concentration of kaempferol-3-(*p*-coumarylglucoside) found in the other species which interferes with the detection of the other analogues.

It has to be kept in mind that the results presented are only based on needles of one tree, collected at one date only. For *L. leptolepis* leaves considerable variations in flavonoid composition were found, dependent on the time of collection [23]. Furthermore, a great variety in leaf flavonoids occurs among different specimen within the species [24].

Experimental

Plant material

Needles of *Larix decidua* Mill. were collected at the arboretum Schovenhorst, Putten, The Netherlands in August 1973. A voucher specimen, no GN 3, was deposited at the Institute for Systematic Botany, University of Utrecht.

Extraction and separation

Freeze-dried needles were extracted with 70% ethanol, chlorophyll and other lipophilic constituents were removed with CCl_4. After concentration the residue was further separated by polyamide column chromatography with water with increasing concentration of methanol, followed either by further separation via repeated banding on Whatman no 1 chromatography paper, or by polyamide column chromatography with chloroform-methanol (9 : 1) (CM 1), again with further purification by paper chromatography.

Identification

The coumpounds were obtained in solution and identified by R_f-values, UV spectral data inclusive spectral shifts, acid hydrolysis/degradation [9], and in a number of cases by alkaline hydrolysis or by peroxide oxidation [10].

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