Flavonoids and Related Compounds in Leaves of Pinaceae, III * *Pinus jeffreyi*

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Nine flavonoids were isolated from leaves of *Pinus jeffreyi* and identified as kaempferol, the 3-glucosides of kaempferol, quercetin, isorhamnetin, laricitrin and syringetin, a 3-glucoside of an A-ring methylated kaempferol derivative, kaempferol-3-(*p*-coumarylglucoside) and kaempferol-3-(ferulylglucoside). Myricetin-3-rhamnoside was found during an earlier investigation.

Introduction

The large genus *Pinus* (90 species) is well-known for polyphenols as far as the woodcomposition is concerned. Needles of amazingly few species, however, have been investigated in some detail. Most knowledge of *Pinus* needles was obtained from general screenings of gymnosperm leaves. Thus, no bi-flavonoids were found in 23 *Pinus* species (73 gymnosperms investigated) [1] and no main C-glycoflavones in 2 species (46 gymnosperms) [2]. Takahaski *et al.* [3] found mainly quercetin and/or kaempferol (in 33 of 42 *Pinus* species), whereas myricetin was only detected in 3 of those 42 species.

In the few species investigated as single object naringenin was found in chloroplasts of *Pinus nigra* [4]. Best investigated are *P. sylvestris* [5–13], *P. sibirica* [14] and *P. contorta* [15] needles. In addition to phenylpropanoids and lignans [8, 11] from *P. sylvestris* needles the flavonoids quercetin- and dihydroquercetin-3-glucoside [8], quercimeritrin (= quercetin-7-glucoside) [6], sylpin (5,6,4'-trihydroxy-3-methoxy-8-methylflavone) [9], acylated flavonol glycosides [12] and 3''-O-*p*-coumaryl-isoquercitrin [13] were isolated. Kowalska [5, 6] suggested, contrary to Sawada's [1] investigations, the presence of two biflavonoids in *P. sylvestris* needles. No confirmation of the occurrence of biflavonoids in pine needles, however, has as yet been obtained. Shumailova [14] isolated a number of catechins (catechin, epicatechin, gallocatechin, epigallocatechin, epigallocatechin gallate and epicatechin gallate) from *P. sibirica* needles. Higuchi and Donnelly [15] found, in addition to some phenylpropanes [16, 17] four acylated flavonol glucosides and 6-methylkaempferol-3-glucoside in needles of *P. contorta*.

Apart from our search for C-glycoflavones [2] no investigations for leaf phenolics have been reported for *P. jeffreyi*.

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Materials and Methods

Needles of *Pinus jeffreyi* Grev. & Balf. were collected at the Pinetum Blijdenstein at Hilversum, The Netherlands the 24th of January 1977. A voucher specimen no GN 12 was deposited at the Institute for Systematic Botany. Only for the isolation of myricetin-3-rhamnoside needles of a previous collection [2], the 20th of September 1973, from the same location were used.

The needles, either fresh or deep-frozen, were extracted with acetone-water and filtered, lipids were removed with petrol ether. After concentration and acidification to pH 2–3, the phenolics were extracted with butanol. The butanol fraction was either used as such or preseparated by polyamide column chromatography eluted with water followed by water-methanol with increasing percentages of methanol. The total butanol extract and the 60% methanol column fraction were further separated by repeated banddchromatography on Whatman No 1 chromatography paper.

The compounds were obtained in solution and identified by R_f-values and colour, UV-spectral data inclusive shifts, acid hydrolysis/degradation [18] and in a number of cases by alkaline hydrolysis.

The butanol extract, preseparated column fractions and purified compounds were also separated or checked for purity by high-performance liquid chromatography (HPLC) on the Dupont 830 chromatograph with a 2.1 ID × 240 mm Zorbax ODS column using a gradient (concave 2, 3%/min.) of 20% ethanol-water to 100% ethanol, both with 0,1% of phosphoric acid at 3000 psi and 50 °C. The flavonoids were detected by their UV absorbance both at 254 and 360 nm. Vitexin was used as internal standard.

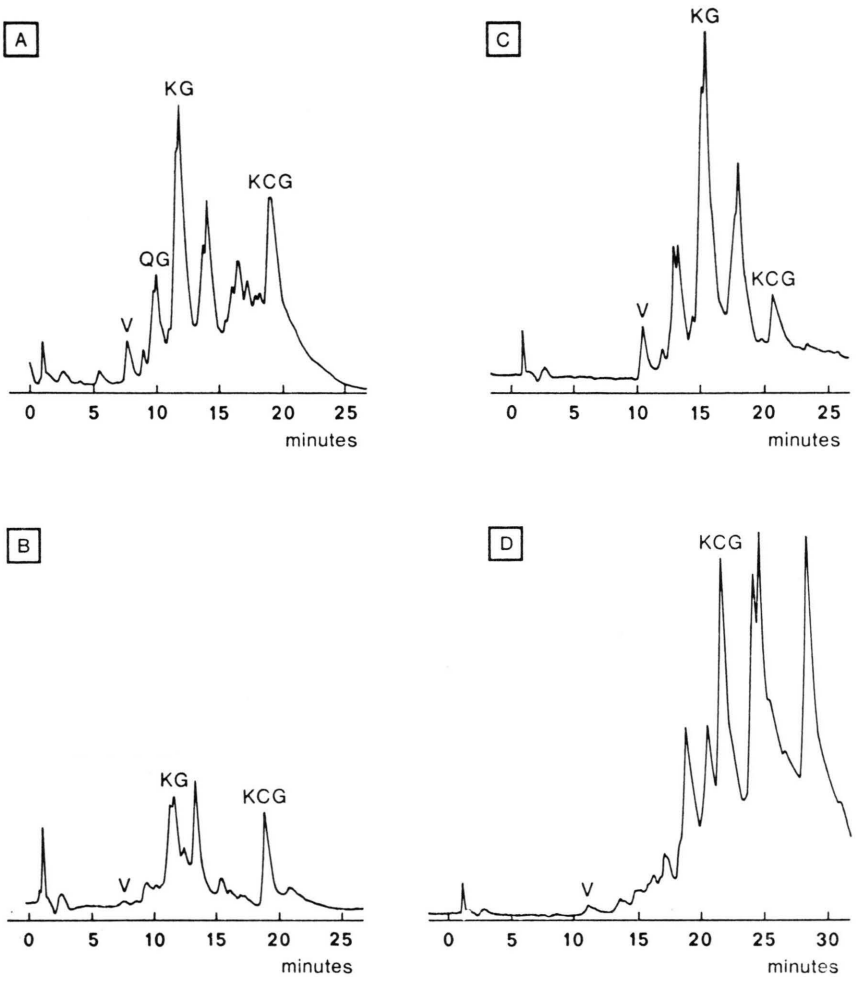

Fig. 1. HPLC separation on a Zorbax ODS column with a gradient of 20% ethanol to 100%, with 0.1% of phosphoric acid, of the *Pinus* butanol extract (A), a butanol extract of *Larix leptolepus* (B), and of the 60% (C) and 80% (D) methanol fractions obtained by polyamide column chromatography of the *Pinus* extract shown under A. V = vitexin, QG = quercetin-3-glucoside, KG = kaempferol-3-glucoside and KCG = kaempferol-3-(*p*-coumarylglucoside).

Results and Discussion

In Fig. I A and B the needle phenolics of a total butanol extract of *P. jeffreyi* (**I A**) were compared with the previously investigated ones of *Larix leptolepis* (**I B**) [19, 20]. Like in wood [21] the *Pinus* flavonoid composition seems much more complex than that of larch needles. On further analysis this appears to be mainly due to the more lipophilic flavonoids (mainly acylated flavonol glycosides) collected in the 80% methanol fraction (compare Fig. **I C** and **D**).

In HPLC analysis co-chromatography was among others found with vitexin (**V**), but this compound was not further identified. At present only main flavonoids from either the total butanol fraction or the 60% methanol fraction were isolated. Eight flavonoids were identified as: kaempferol, the 3-glucosides of kaempferol (KG), quercetin (QG), isorhamnetin, laricitrin (= 3'-methylmyricetin), and syringetin, and the 3-(*p*-coumarylglucoside)- (KCG) and 3-(ferulylglucoside)- of kaempferol. In a previous isolation, from needles collected in September instead of January, myricetin-3-rhamnoside was identified, but this compound was not detected amongst the main flavonoids of the January needles.

Partly identified was a 3-glucoside of a somewhat lipophilic kaempferol derivative. The occurrence of *p*-hydroxybenzoic acid as a degradation product suggests an A-ring methylated kaempferol glucoside probably identical with the 6- methylkaempferol-3-glucoside recently identified in needles of *P. contorta* [15], with which it co-chromatographs. A diglycoside of the same aglycone was also present.

Not identified remained a number of blue fluorescent glucosides and esters, two flavanone glucosides and an acylated flavanone glucoside.

The general flavonoid pattern found in *P. jeffreyi* needles appears rather similar to that found in other Pineceae like larch and *Cedrus* [20], although the diglycosides seem to occur in a much lower concentration than in the other species. Possibly, the time of needle collection may be hold responsible. For *L. leptolepis* needles a large influence of the season was shown on the needle flavonoid pattern [22].

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