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PHENOLICS FROM LARIX NEEDLES. X. FLAVONOIDS OF L. GMELINII

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SUMMARY

Eight flavonoids were isolated and identified as vitexin and its glucoside (8-C-diglucosylapigenin), the 3-glucosides of kaempferol, myricetin, 3′-methylmyricetin and syringetin, the 3-rutinoside of the latter and the 3-(*p*-coumaroylglucoside) of kaempferol. A great similarity was found in the flavonoid patterns of needle extracts of the *Larix* species hithertoo investigated.

1. INTRODUCTION

Investigations on Larch needles were started more or less simultaneously at Irkutsk (MEDVEDEVA et al. 1971, 1972a, b, c, 1973, TJUKAVKINA et al. 1974) and Utrecht (NIEMANN 1969, 1971, 1972a, 1973a, b, 1974, NIEMANN & BEKOOY 1971). Whereas Larch heartwood is rather simple in flavonoid composition as compared with other Pinaceae (HARBORNE 1967), in the needles a surprisingly rich array of flavonoids with a very complete hydroxylation/methoxylation pattern was found (MEDVEDEVA et al. 1974, NIEMANN 1974). Only three species, *Larix sibirica*, *L. laricina* and *L. leptolepis* were investigated in detail. To obtain a more complete picture extension of the series with more *Larix* species for at least the main needle flavonoids was indicated. In this connection some flavonoids of *L. decidua* (NIEMANN 1975) and of *L. gmelinii* needles were isolated and identified.

2. MATERIAL AND METHODS

Needles of *Larix gmelinii* (Rupr.) Kuzeneva were collected at the Arboretum Schovenhorst, Putten, The Netherlands in August 1973. A voucher specimen no. GN 4 was deposited at the Institute for Systematic Botany, University Utrecht.

Freeze-dried needles were extracted with acetone-water; chlorophyl and other lipophilic constituents were removed with light petrol. After concentration, the solution was further separated by extraction with ether followed by butanol (TISSUT & EGGER 1972). Ether and butanol fractions were purified by repeated banding on Whatman no 1 chromatography paper and/or on silica thin layer.

The compounds were obtained in solution and identified by R_f values, UV

spectral data inclusive spectral shifts, acid hydrolysis/degradation (NIEMANN 1972b), and in a number of cases by alkaline hydrolysis or peroxide oxydation (CHANDLER & HARPER 1961).

3. RESULTS AND DISCUSSION

Comparison of one- and two-dimensional chromatograms of total needle extracts of *L. gmelinii* with the previously investigated *L. laricina* and *L. leptolepis* sheets indicated a rather high similarity. However, although most spots appeared present in all three species the relative concentrations were often quite different. Thus, *L. gmelinii* needles were, for instance, very rich in myricetin derivatives when compared with *L. leptolepis*. This aspect is also apparent when looking at the nature of the compounds finally isolated since the method used restricted identification to *main* flavonoids. Nine flavonoids were isolated of which eight were identified as kaempferol-3-glucoside and -3-(*p*-coumaroyl-glucoside), myricetin-3-glucoside, 3'-methylmyricetin-3-glucoside, syringetin-3-glucoside and -3-rutinoside, and vitexin and its glucoside (8-diglucosylapigenin). All compounds have been isolated before from at least one of the larch species mentioned. 3'-Methylmyricetin glycosides up to now have only been isolated from larch species (NIEMANN 1972a, 1973b; TJUKAVKINA et al. 1974). The latter authors proposed the name laricitrin for the aglycone.

The ninth flavonoid, also found in *L. leptolepis* leaves, was only partly identified. Its UV spectrum (maximum in ethanol: 267 327 353 nm; shift: NaAc 0, NaOH + 38, $AlCl_3$ + 50) points to a 7-(and 4'?-) substituted kaempferol derivative. The maximum at 327 nm might indicate the introduction of an 8-hydroxyl group. Acid hydrolysis/degradation produced *p*-hydroxybenzoic acid and glucose, confirming the kaempferol character for the B-ring part. R_f values, and especially that in phenol, suggest the introduction of one or more methyl substituents probably in the A-ring part of the molecule ($R_f \times 100$ on paper – between brackets that for kaempferol-3-glucoside – : TBA 57 (67), 15HAc 44 (43), H_2O 14 (12), Phenol 89 (68); on polyamide: WEMA 51 (36)).

Because of the suspected 8-hydroxyl (or -methoxyl?), isolation in higher quantity gains importance in connection with the supposed evolutionary status of flavonoid characters (HARBORNE 1967). Larch needles have been found rich in flavonols and C-glycoflavones. Flavanones (NIEMANN 1974) and leucoanthocyanidin (leucocyanidin in *L. leptolepis* needles – Niemann, not published) were found as well. Apart from these "primitive characters", however, the presence of flavonoids with methylated galloyl units (3'-methylmyricetin, syringetin) gives larch a special place among the Pinaceae. 8-Hydroxylation, as well as O-methylation considered as a "gain mutation", would add in this aspect.

Comparing *L. gmelinii* needles with those of other larch species one of the most obvious features seems the absence of quercetin derivatives, and especially of isorhamnetin glycosides, among the isolated compounds. As mentioned, this may partly be explained by the method of isolation used with which minor flavonoids were neglected. Two-dimensional chromatography indeed indicated

the possible occurrence of isorhamnetin-3-glucoside, present however, as a minor constituent only.

In general the species investigated seem to possess similar enzyme systems in flavonoid biosynthesis. However, whereas kaempferol glycosides are main flavonoids in all cases, for polyhydroxylation of the B-ring there seems to be a preference which differs with different species. Thus, species were found rich in quercetin derivatives (*L. decidua*) or rich in myricetin (*L. gmelinii* and to a lesser extent *L. laricina*), or they belong to an intermediate type (*L. leptolepis*). 3-Glycosylation with glucose or rhamnosylglucose is the main pattern in all larch species investigated. For the C-glycoflavones, remarkably occurring as major compound in six larch species but not in other Pinaceae investigated (NIEMANN & MILLER 1974), the sugar attached to the 8-C-glucose varies with the species.

Although care was taken to collect needles at the same day, from trees growing in the same area, environmental factors may, of course, not be excluded.

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