Flavonoids and Related Compounds in Leaves of Pinaceae. II*. *Cedrus atlantica* c. v. Glauca

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(Z. Naturforsch. **32 c**, 1015–1017 [1977]; received September 12, 1977)

Cedrus atlantica, Pinaceae, Flavonoids

Ten flavonol glycosides were isolated from leaves of *Cedrus atlantica*. Among the six aglycones the rarely occurring syringetin and laricitrin were found in addition to kaempferol, quercetin, isorhamnetin and myricetin. 3-Glycosylation with glucose and/or rutinose was the main pattern, whereas acylation with *p*-coumaric acid occurred for kaempferol- and isorhamnetin-3-glucoside.

Introduction

Little is known of leaf flavonoids in most Pinaceae species. The only genus of which a number of species are fairly wellknown, *Larix*, appeared to contain a rich array of flavonoids in the needles [1]. This was quite surprising since *Larix* is comparatively very simple in the flavonoid composition of its wood [2]. Within the genus all larch species investigated were found very similar in needle flavonoids. The single species of the closely related genus *Pseudolarix*, *P. amabilis*, however, was quite different in this aspect [3]. Within the Pinaceae, *Larix*, *Pseudolarix* and *Cedrus* belong to the subfamily Laricoideae. It became of interest to know more of *Cedrus* in order to evaluate a possible interrelationship from a chemotaxonomical view. Of the four *Cedrus* species only *C. deodora* had been investigated for leaf flavonoids in a general screening of coniferae leaves by Takahashi *et al.* [4]. The presence of two glycosides was demonstrated, but no flavonoids were identified. In our laboratory the opportunity was given to investigate leaves of *Cedrus atlantica* c.v. Glauca during undergraduate courses. The results of this work are represented here.

Material and Methods

Needles of *Cedrus atlantica* (Endl.) Carr. c.v. Glauca were collected at the Pinetum Blijdenstein, Hilversum, The Netherlands the 24th of January 1977. A voucher specimen no GN 13 was deposited at the Institute for Systematic Botany, University of Utrecht.

The needles, either fresh or deep-frozen, were extracted with acetone-water, filtered and the acetone solution was concentrated and extracted with butanol after removal of lipids with light petrol. The butanol fraction was either used as such or preseparated by column chromatography on polyamide, eluted with water followed by water-methanol with increasing percentages of methanol. Further separation was obtained by repeated band-chromatography on Whatman no 1 paper and/or silica thin layer. The compounds were obtained in solution and identified by R_F values and colour, UV spectral data inclusive shifts, acid hydrolysis/degradation [5] and in a number of cases by alkaline hydrolysis or peroxide oxidation [6]. Products of hydrolysis and/or degradation were identified by comparison with adequate reference substances both on paper and thin layer and, for sugars and some aromatic acids, also by means of gas liquid chromatography (GLC). For GLC sugars were silylated with hexamethyldisilazane/trimethylchlorosilane in pyridine at room temperature and chromatographed on 3% SE 30, temperature-programmed from 100 – 270 °C at 2.5°/minute. Aromatic acids were methylated with 14% boron trifluoride in methanol [7] and chromatographed on a column with 10% Apiezon-L on Chromosorb W, isotherm at 140 °C.

Total butanol extracts, 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% phosphoric acid at 3000 psi and 50 °C (Program II). This system gave a better resolution than our previous one [8] with a 4%/min gradient of 45 – 100% methanol with 0.1% acetic acid under otherwise similar conditions (Program I). Vitexin was mainly used as internal standard. Flavonoids were detected by their UV absorbance both a 254 and 360 nm.

Results and Discussion

In Figs 1 and 2 typical HPLC chromatograms for total butanol extracts are shown. To include a comparison with older *Pseudolarix* extracts analysed with a previous program (I, Fig. 1 a) an analysis of *Larix leptolepis* with the same program has been added (Fig. 1 b) in addition to the chromatograms of *L. leptolepis* and *Cedrus atlantica* (Fig. 2 a and b). From these pictures only little information can be obtained for the flavonol-3-glycosides (retention time around 15 min) since they are either not or only badly resolved. The more lipophylic flavonoids, like for example the acylated flavonol

* Part I in this series: G. J. Niemann, Z. Naturforsch. **30 c**, 550 [1975].

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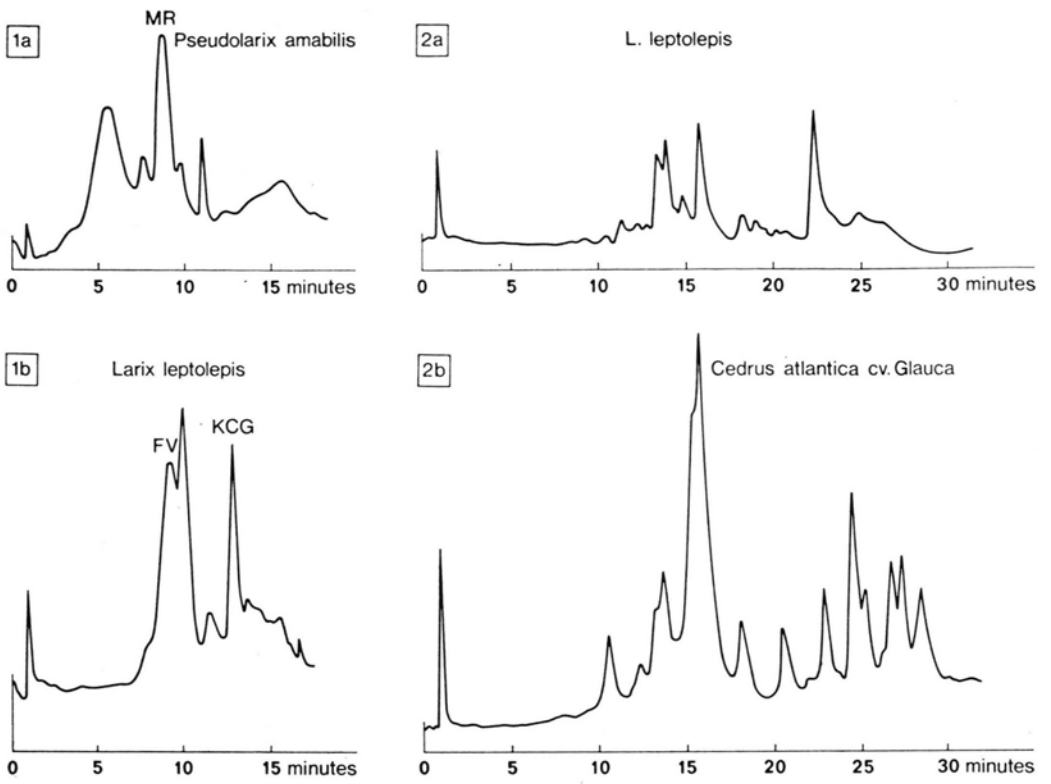

Fig. 1 and 2. Separation of plant extracts on a 24×0.21 Zorbax ODS column with a gradient of methanol-water with 0.1% acetic acid (1a and b), and with a gradient of ethanol-water with 0.1% of phosphoric acid (2a and b). MR = myricetin-3-rhamnoside, FV = flavonol-3-glycosides with isorhamnetin-3-glucoside and/or kaempferol-3-glucoside (KG) as main constituents, and KCG = kaempferol-3-(*p*-coumaryl-glucoside).

glycosides are much better separated. In this area (RT 18 and more) a much more complex flavonoid composition is found for *C. atlantica* than for both *P. amabilis* and *L. leptolepis*. On further analysis most 3-glycosides were found in the 40% and 60% MeOH column fractions and the flavonoids with higher HPLC RT in the 100% one.

Repeated bandchromatography of the total butanol extract gave a number of flavonoids of which kaempferol-3-glucoside (KG) and laricitrin-3-glucoside (= 3′-methylmyricetin-3-glucoside, LG) and the acylated glycosides kaempferol-3-(*p*-coumaryl-glucoside and isorhamnetin-3-(*p*-coumarylglucoside) were isolated. Acylation of the same 3-glycosides, KG and isorhamnetin-3-glucoside, also occurred with another acid which has not yet been identified. Two other 3-glycosides were present in too small a concentration for complete identification but were tentatively identified as the 3-glucoside and 3-rutinoside of quercetin.

Analysis of the 60% MeOH column fraction in addition to KG and LG also gave the 3-glucosides of isorhamnetin and myricetin and the 3-rutinosides of kaempferol, isorhamnetin, laricitrin and syringetin.

The, as yet incomplete, analysis of *C. atlantica* leaves reveals a striking resemblance with the flavonoid composition found in different larch species. In both cases 3-glycosylation occurs, leading to glucosides and rutinosides. For the flavonols the same complete hydroxylation/methylation B-ring pattern is found, leading to such rare compounds as syringetin (up to now only found in 5 plant species outside the Pinaceae) and laricitrin (in only 2 other species) [9]. Acylation of flavonol glycosides even seems more outspoken in *Cedrus* than in *Larix*. Flavones and flavanones were hithertoo not identified, but spectral evidence of some low-concentration fractions indicate the presence of one or more flavanones.

As far as leaf flavonoids are concerned, it thus seems that species in the genera *Larix* and *Cedrus* are more related than *Pseudolarix* and these two. *C. atlantica* is the most complex species in this aspect.

I am indebted to Judith Koerselman-Kooy for skilful assistance, to 18 undergraduate students for their enthousiastic contribution and to my colleagues Hans van Genderen and Frans Warnaar for aid with improvement of flavonoid-HPLC and with sugar-GLC, and with aromatic acid-GLC respectively.

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