Phenolics from *Larix* Needles. XV. High-Performance Liquid Chromatography of *L. gmelinii* Flavonoids*

Gerard J. Niemann

Botanisch Laboratorium, Rijksuniversiteit Utrecht, Lange Nieuwstraat 106, Utrecht, The Netherlands

Z. Naturforsch. **35 c,** 514–515 (1980);
received January 24, 1980

Larix gmelinii, Pinaceae, Flavonoids, High-Performance Liquid Chromatography

From needles of *Larix gmelinii* sixteen flavonoids were isolated and identified. The main flavonoids had been analyzed before, eight minor ones were identified as quercetin-3-glucoside, isorhamnetin-3-glucoside and -3-arabinoside, a somewhat lipophilic kaempferol-3-glucoside derivative, apigenin-7-glucoside, laricitrin-3-rutinoside and laricitrin- and syringetin-3-(*p*-coumarylglucoside).

Introduction

Leaves of Pinaceae species have been comparatively little investigated for phenolic constituents [1] although in general they appear a rich source of a variety of flavonoids. *Larix gmelinii* has been searched for some of its major flavonoids [2, 3]. It showed a needle flavonoid pattern similar to other larch species [1], having among others the rare flavonols laricitrin (3′-methylmyricetin) and syringetin and the same glycosylation type. Surprisingly, however, quercetin derivatives were not found among the major *L. gmelinii* needle flavonoids although some indication of their presence was obtained [3]. Many more flavonoids were present in comparatively low concentration and a number of those have now been isolated and are described.

Materials and Methods

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

Extraction and isolation. Freeze-dried needles were extracted with 70% ethanol; from this extract chlorophyll and other lipophilic compounds were removed with carbon tetrachloride. The residual ethanolic fraction was used for high-performance liquid chromatography (hplc) or concentrated and fractionated by polyamide column chromatography using stepwise elution with water-methanol mixtures with increasing concentration of methanol. The 60% and 80% methanol eluates were subsequently separated by polyamide column chromatography with chloroform-methanol 9:1 as eluens and purified by paper chromatography (pc). The aqueous and the 40% methanol fraction, containing esters and glycosides of the aromatic hydroxy acids, and vitexin and its glycosides respectively, were investigated as such using pc, tlc and hplc.

The compounds were obtained in solution and identified by R_f and/or t_r (hplc retention time); by their color under uv as such, with ammonia and after spraying with flavone reagent [4]; by uv spectral data inclusive spectral shifts obtained after addition of sodium acetate followed by sodium hydroxide, of aluminum chloride followed by diluted hydrochloric acid and of sodium acetate followed by boric acid; by acid hydrolysis/degradation [5, 6]; and in a number of cases by alkaline hydrolysis. In most cases the identity was confirmed by co-chromatography with the original compound.

Hplc was carried out with a Dupont chromatograph, using a 4.2 × 240 mm Zorbax ODS column eluted with a gradient (concave 2, 45–100%) of methanol-water with 0.1% of phosphoric acid at 50 °C, a pressure of 1100 psi (7500 kPa, flow around 0.5 ml/min) and a rate of 3%/min. The compounds were detected by uv at 254 and 360 nm. Vitexin was used as internal standard.

Results and Discussion

In addition to the major flavonoids already isolated by repeated paper chromatography [3], column chromatography also yielded a number of flavonoids present in lower concentration. Identified were isorhamnetin-3-glucoside and -3-arabinoside, quercetin-3-glucoside, apigenin-7-glucoside, laricitrin-3-rutinoside and -3-(*p*-coumarylglucoside) and syringetin-3-(*p*-coumarylglucoside). The 3-glucosides of a kaempferol and a syringetin derivative with some-

* Part XIV: G. J. Niemann and W. J. Baas, Z. Naturforsch. **33 c,** 780 (1978).

Reprint requests to Dr. G. J. Niemann.
0341-0382/80/0500-0514 $ 01.00/0

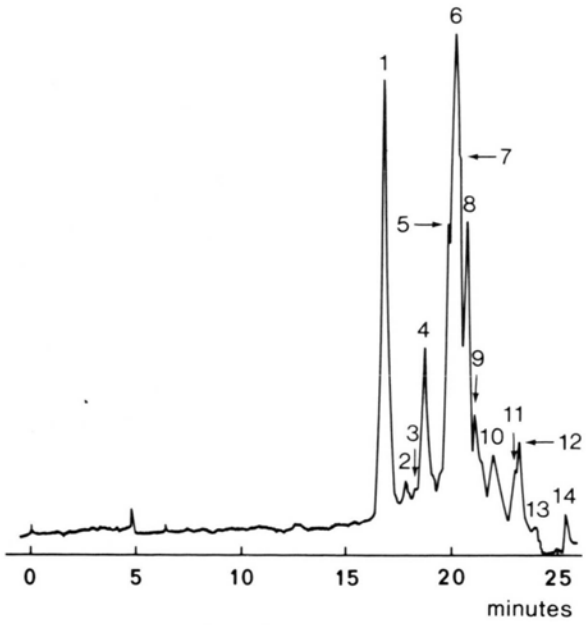

Fig. 1. Hplc separation of the ethanol extract of *Larix gmelinii* needles on a Zorbax ODS column eluted with a gradient of methanol-water acidified with 0.1% of phosphoric acid. 1: combination of vitexin, its (8-)glycoside and myricetin-3-glucoside, 2 and 3: unknown, 4: quercetin- and laricitrin-3-glucoside, 5: apigenin-7-glucoside, 6: kaempferol-3-glucoside, 7: isorhamnetin- and syringetin-3-glucoside, 8: unknown, 9: a lipophilic kaempferol-3-glucoside incompletely separated from isorhamnetin-3-arabinoside (shoulder), 10: laricitrin-3-(*p*-coumarylglucoside), 11: kaempferol-3-(*p*-coumarylglucoside), 12: syringetin-3-(*p*-coumarylglucoside), 13: unknown and 14: co-chromatographes with naringenin-5?-(*p*-coumarylglucoside).

what more lipophilic properties than kaempferol- and syringetin-3-glucoside, were only partly identified. Acid degradation [5] gave *p*-hydroxybenzoic acid and syringic acid respectively, indicating a flavonol B-ring structure similar to that of kaempferol and syringetin. The R_f values of the two flavonol glucosides suggest A-ring methylation. For the kaempferol derivative all data were very similar to, but not quite identical with, 6-methylkaempferol-3-glucoside, recently isolated from pine needles [7, 8].

The localization of the flavonoid peaks in a hplc chromatogram of the total needle extract occurred by co-chromatography with the isolated compounds and is shown in Fig. 1. As expected [9, 10] complete resolution of the *Larix* extract by hplc could not be obtained. Peaks like no's 1, 4, and 7 (Fig. 1) still represent a combination of two or more compounds. Peak 14 co-chromatographes with a compound isolated from *L. decidua* and at the time provisionally identified as naringenin-5-(*p*-coumarylglucoside) [11], but this compound was not isolated in any quantity from *L. gmelinii* needles.

Acknowledgement

The assistance of Mrs. Judith Koerselman-Kooy is greatfully acknowledged.

[1] G. J. Niemann, Acta Bot. Neerl. **28,** 73 (1979).
[2] N. A. Tjukavkina, S. A. Medvedeva, and Z. Ivanova, Khimia drevesiny (Riga) **16,** 93 (1975).
[3] G. J. Niemann, Acta Bot. Neerl. **24,** 65 (1975).
[4] H. C. Dass and G. M. Weaver, J. Chromatogr. **67,** 105 (1972).
[5] G. J. Niemann, J. Chromatogr. **74,** 155 (1972).
[6] G. J. Niemann, J. Chromatogr. **170,** 227 (1979).
[7] R. Higuchi and D. M. X. Donnelly, Phytochemistry **17,** 787 (1978).
[8] G. J. Niemann, Z. Naturforsch. **33 c,** 777 (1978).
[9] G. J. Niemann and J. W. Koerselman-Kooy, Planta Med. **31,** 297 (1977).
[10] G. J. Niemann and J. van Brederode, J. Chromatogr. **152,** 523 (1978).
[11] G. J. Niemann and W. J. Baas, Z. Naturforsch. **33 c,** 780 (1978).