Acta Bot. Neerl. 21(5), October 1972, p. 549–552

PHENOLICS FROM LARIX NEEDLES IV. CONSTITUENTS OF L. LARICINA

G. J. NIEMANN

Botanisch Laboratorium, Utrecht

SUMMARY

Four phenolics were positively identified in *Larix* needles as vitexin, syringetin-3-glucoside, *p*-hydroxybenzaldehyde, and vanillin. A fifth compound was tentatively identified as a 3′ substituted myricetin-3-glucoside.

1. INTRODUCTION

In previous papers the isolation was reported of the glucosides of *p*-hydroxybenzoic-, vanillic- and *p*-coumaric-acid (NIEMANN 1969) and of the flavonoids kaempferol-3-glucoside, isorhamnetin-3-glucoside, xylosylvitexin and glucosylxylosylvitexin (NIEMANN & BEKOOY 1971) from needles of *Larix laricina.* Recently, the hydroxy-acid glucosides have also been found in needles of *L. sibirica* (MEDVEDEVA *et al.* 1971). Kaempferol-3-glucoside has been isolated from needles of *L. kaempferi* Sargent (TAKAHASHI *et al.* 1960) and from needles of *L. sibirica* (MEDVEDEVA *et al.* 1972).

Continuation of the investigation of needles of *L. laricina* led to the isolation of some flavonoids and aldehydes as reported here.

2. MATERIAL AND METHODS

Needles of *L.laricina* (Du Roi) K. Koch were collected at the Arboretum, University of Wisconsin, Madison, September 1968, and at the Gimborn Arboretum, State University Utrecht, September 1969.

Freeze-dried needles were extracted with ethanol. The extract was dried and separated by polyamide column chromatography or by $NaHCO_3$ - BuOH partition, next by silica column chromatography. The compounds were further purified by banding on silica TLC and/or paper. Extraction procedures have been described more extensively elsewhere (NIEMANN 1971).

3. RESULTS AND DISCUSSION

Two aldehydes were isolated from the 10% ethanol fraction of a polyamide column and identified by colour reactions, UV spectral data and cochromatography with the authentic compounds (three solvents, thin layer and paper) as *p*-hydroxybenzaldehyde and vanillin.

Of the three flavonoids isolated only two were positively identified as vitexin

and syringetin-3-glucoside. The quantity of the third compound, code number N 25C, was not sufficient for a complete elucidation of its structure. The chromatographic and UV spectral data of the flavonoids are summarized in *tables 1* and *2* respectively. Because of its similarity to syringetin-3-glucoside, data about the previously isolated isorhamnetin-3-glucoside (NIEMANN & BEKOOY 1971) are included as well. The two compounds separated well on paper with tert.butanol/acetic acid/water (3:1:1) (TBA) or on polyamide thin layer with water/ethanol/methylethylketone/acetylacetone (55:20:20:5) (WEMA) (solvent from TISSUT & EGGER 1972), but not with the other solvents listed in *table 1*.

The identification of vitexin was based on comparison with the authentic compound. Syringetin-3-glucoside was identified by its spectral and chromatographic properties and by the products of acid hydrolysis. Acid hydrolysis yielded only one sugar, identified as glucose, and an aglycone resembling isorhamnetin except in its R_f value in phenol. The latter already pointed to syringetin (HARBORNE 1967). More decisive evidence, however, was obtained from the fact that the aglycone on hydrolysis partly degraded with syringic acid as one of the products. The position of the sugar in the molecule was determined from the spectral shifts before and after hydrolysis.

Acid hydrolysis of flavonoid N 25C produced glucose and an aglycone with an ortho-dihydroxyl configuration in the B ring (borate shift). Pyridine/HBr

Table 1. *Larix* flavonoids, chromatographic results

	R_f (× 100)								
	Paper								Polyamide
Compound	BeAW[1]	TBA	BuAW	BuEtW	15HAc	Phenol	Forestal	H_2O	WEMA
Larix vitexin	31		59	44	27	63	80	7	42
vitexin[2]	30		58		25	64	82	7	
Isorhamnetin-3-glucoside	50	58	77	52	43	76	86	19	45
Syringetin-3-glucoside	50	52	67	53	42	83	85	16	48
Syringetin	63		73		4	76	51		
Isorhamnetin[3]	60		73		4	69	50		
N 25C	35		59	45	31	56	84	10	44
N 25C - Acid hydrolysate	27		57		4	37	40		
Myricetin	14		50		4	11	28		
Quercetin	29		69		5	32	43		

[1] BeAW = benzene/acetic acid/water (125:72:3), TBA = tert. butanol/acetic acid/water (3:1:1), BuAW = butanol-1/27% acetic acid (1:1), BuEtW = butanol-1/ethanol/water (4:1:2,2), 15 HAc = 15% acetic acid, WEMA = water/ethanol/methylethylketone/acetylacetone (55:20:20:5).

[2] From the collection of Dr. M. K. Seikel.

[3] Obtained by courtesy of Dr. T. Mabry.

Table 2. UV spectra of *Larix* flavonoids

Compound	Absorption maxima, λ_{max} in nm				
	In EtOH	+ NaOAc	+ NaOEt	+ NaOAc + H_3BO_3	+ $AlCl_3$
Larix vitexin	269,307^s,334	278,298^s,378	277,319^s,388	271,300,337	277,302,340,381
vitexin[2]	271,308.5,335	279.5,300^s,366	283,323,391		280,304.5,342,383
Isorhamnetin-3-glucoside	252,263^s,292^s,356	250,268,292^s,360	253,268,293^s,362	251,265^s,292^s,360	263,303,358,400
Syringetin-3-glucoside	251,264^s,303^s,360	251,268,308^s,368	250,264,371	251,264^s,304^s,361	266,303,361,397
Syringetin	253.5,263^s,305,372	254,263,325,377	dec.	253,270^s,377	263,305^s,356^s,425
Isorhamnetin[3]	255,266^s,307^s,372	256,325,378	dec.	253,265^s,305^s,375	263.5,269,358,426
N 25C	253^s,264,309^s,360	253^s,271,309^s,366	282.5,325,408[1]	266,307,382	273,306,360,400
N 25C - Acid hydrolysate	254.5,265^s,300,373	253.5,264^s,300^s,377	dec.	255,297^s,327,380	268,299^s,354,428

[1] decomposes slowly. [2] From the collection of Dr. M. K. Seikel. [3] Obtained by courtesy of Dr. T. Mabry.

degradation gave myricetin. One of the degradation products on acid hydrolysis had properties of an ortho-dihydroxy benzoic acid similar to, but not identical with, protocatechuic acid. Thus, a 3′ substituted myricetin glucoside seems indicated. The spectra indicate a 3 position for the sugar. The most likely substituent at the 3′-position is a methyl group making N 25C identical with 3′-methylmyricetin-3-glucoside, but this still is to be proved.

ACKNOWLEDGEMENT

The assistance of Judith Koerselman-Kooij and Betty Boonzaaijer-van Dijk is appreciated.

REFERENCES

HARBORNE, J. B. (1967): *Comparative biochemistry of the flavonoids.* Academic Press, London and New York.

MEDVEDEVA, S. A., N. A. TJUKAVKINA & S. Z. IVANOVA (1971): Glucosides of phenolic acids from needles of Larix sibirica. *Khim. Prir. Soedin. (Tashkent)* 844.

—, — & — (1972): Astragalin from needles of Larix sibirica. *Khim. Prir. Soedin. (Tashkent)* 123.

NIEMANN, G. J. (1969): Phenolic glucosides from needles of Larix laricina. *Phytochem.* **8**: 2101–2103.

— (1971): Phenolic constituents from Larix needles III: Benzoic and cinnamic acids of L. leptolepis. *Acta Bot. Neerl.* **20**: 397–400.

— & R. BEKOOY (1971): Flavonoid constituents from Larix needles. *Phytochem.* **10**: 893.

TAKAHASHI, M., K. ITO, A. MIZUTANI & K. ISOI (1960): Chemical constituents of the plants of Coniferae and allied orders XLIII. Distribution of flavonoids and stilbenoids of Coniferae leaves. *J. Pharm. Soc. Japan* **80**: 1488–1492.

TISSUT, M. & K. EGGER (1972): Les glycosides flavoniques foliaires de quelques arbres, au cours du cycle végétatif. *Phytochem.* **11**: 631–634.