PHENOLIC CONSTITUENTS FROM LARIX NEEDLES III: BENZOIC AND CINNAMIC ACIDS OF L. LEPTOLEPIS

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SUMMARY

Six phenolics were isolated from needles of *Larix leptolepis* and identified as *p*-coumaric acid, ferulic acid, *p*-hydroxybenzoic acid, protocatechuic acid, vanillic acid and syringic acid. Different extraction procedures indicated, however, that these compounds only occurred in the needles in a bound form. The finding of free acids had to be attributed to an artifact in the extraction procedure.

1. INTRODUCTION

In previous work on benzoic- and cinnamic acid type constituents of *Larix* needles (NIEMANN 1969) the occurrence of low concentrations of free acids (e.g. vanillic acid) was noticed (not published). The presence of glycosides of these acids suggested the possibility that an artifact in the extraction procedure could be responsible for the occurrence in the free state. In view of a possible physiological significance an investigation of the presence in free or bound form (or in both) was indicated. Therefore, two extraction procedures were used, in one of which special care was taken to prevent hydrolysis.

2. MATERIAL AND METHODS

Needles from *L. leptolepis* (Sieb. et Zucc.) Gord. were collected at Austerlitz (The Netherlands) in July 1970.

In Method I fresh needles were immersed in petrol ether directly after picking and ground in a Wareing Blendor. The needles were extracted successively with petrol ether, chloroform, chloroform/methanol 2% (CM2), chloroform/methanol 10% (CM 10) and methanol. The extracts were evaporated to dryness *in vacuo* and the dry residues were investigated by one- and two-dimensional paper chromatography.

In method II the needles were directly frozen with liquid nitrogen. The frozen needles were kept in the deep-freezer until they could be frozen to dryness. The dry needles were ground in a Beco mill and subsequently extracted with the same solvents as in method I. Only ethanol 96% was used as last solvent instead of methanol because in other work on phenolics a slow decomposition of some of the flavonoids in methanolic solution was noticed (H. 't Hart, unpublished).

[1] For number I and II of this series see: *Phytochem.* **8**, 2101 (1969) and *Phytochem.* **10** (1971).

Again, the extracts were concentrated *in vacuo*. For both the I and II extracts paper and thin-layer chromatographic surveys were carried out with the solvent pairs: butanol-1/27% acetic acid (1:1) (BuAW) and 15% acetic acid (HAc); BuAW and water saturated phenol and BuAW and butanol-1/ethanol/water (4:1:2.2) (BuEtW) on paper Whatman no. 1; with chloroform/acetone (6:4) and chloroform/methanol 15% on silica thin-layer and, one-dimensionally, with isopropanol/ammonia/water (8:1:1) (IAmW) on Schleicher and Schull no 2045b paper or cellulose thin layer. The compounds were detected by viewing under UV light of 254 or 360 nm, both without and with ammonia vapour, and by spraying with *p*-nitrobenzene-diazonium tetrafluoroborate (DNA), diazotized sulfanilic acid (DSA) (both oversprayed with 20% aq. sodium carbonate) or with ferric chloride 1%. Afterwards, the extracts resulting from series I were further subdivided by successive extraction of an aqueous butanol solution with 2% sodium acetate, with saturated sodium bicarbonate and with 20% aq. sodium carbonate (STROHL & SEIKEL 1965). The alkaline solutions were acidified with hydrochloric acid and extracted with butanol. The resulting butanol extracts A (from NaAc, containing the stronger acids), C ($NaHCO_3$, mainly flavonoids), D (Na_2CO_3, very weak acids, phenols), and B (the remaining butanol solution, chlorophyl, lipoids a.o.) were concentrated and again investigated by paper chromatography.

3. RESULTS AND DISCUSSION

Fresh needles contained about 67 (method I) to 63 or 64% of water (method II, a slight difference was found between dry needle weight and dry weight of total extractives + residue). In method I water was retained in the column during the petrol ether, chloroform, and CM 2 extractions, it was found in the CM 10 fraction as a separate layer. In total 13.1% (I) and 13.2% (II) of the fresh weight was recovered as dry extractable material, air-drying left 20 to 24% unextractable residue.

Extraction according to I resulted in a number of DNA- and DSA-positive compounds in the CM 2 and CM 10 extracts. Afterwards the main portion of these compounds was found in the butanol A extracts. Extraction was not complete, however, and some was left in the butanol C and D extracts as well. Six phenolics were identified (see *table 1*) as *p*-coumaric acid, ferulic acid, *p*-hydroxybenzoic acid, protocatechuic acid, vanillic acid, and syringic acid.

When the needles were frozen to dryness according to II, however, none of the mentioned acids could be detected. Only after acid or alkaline hydrolysis free acids were found, mainly in the hydrolysate of the CM 10 fraction. It thus appears that the occurrence of these free acids in needle extracts of *L. leptolepis* has to be attributed to an artifact in the extraction procedure. Most probably the compounds occur in glycosidic bound form as found previously for *p*-coumaric, vanillic and *p*-hydroxybenzoic acid in *L. laricina* needles (NIEMANN 1969) or as esters. Active degrading enzymes might explain the high yield of the free acids in method I in which water was retained for a comparatively long time.

Table 1. Phenolic acids from *Larix leptolepis*[1]

	Colour reactions[2]						R_f values (× 100) on paper[3]											
Compound	254 nm	360 nm	$+NH_3$	DNA	DSA	$FeCl_3$	BuAW		BuEtW		HAc		IAmW		Phenol		BeAW	
p-coumaric acid	abs.	B	B	dB	R	–	89	88	73	72	57/75	57/75	34/39	34/38	67	67	79	79
ferulic acid	B	B	iB	B	P	–	87	88	79	78	50/73	53/74	24/32	23/31	79	79	83	83
p-hydroxybenzoic acid	abs.	–	–	R	OY	–	91	90	80	81	71	72	26	26	66	67	75	73
vanillic acid	VB	–	–	P	O	–	87	88	74	74	68	67	20	20	77	78	82	82
protocatechuic acid	VB	–	VB	PW	PO	dGy	77	75	71	71	64	65	8	9	32	32	57	58
syringic acid	VB	–	–	B	RO	–	84	84	72	72	67	67	15	15	82	82	78	78

[1] See the chapter on material and methods for the abbreviations used for the solvent systems and spray reagents, BeAW = benzene/acetic acid/water (125:72:3)

[2] abs. = absorbent, B = blue, V = violet, i = intens, d = dark, R = red, P = purple, OY = orange yellow, W = white, O = orange, Gy = grey

[3] The first value gives the R_f of the *Larix* compound, the second that of the authentic sample. For the cinnamic acids both the values for the cis- and trans compound are given on separation in the more aqueous systems.

ACKNOWLEDGEMENT

The assistance of Truus Keijzer and Judith Kooy is highly appreciated.

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