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SHORT COMMUNICATION

PHENOLIC GLUCOSIDES FROM NEEDLES OF *LARIX LARICINA*

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Abstract—Three phenolic glucosides were isolated from needles of *Larix laricina* and identified as the β-glucosides of vanillic acid and *p*-coumaric acid and as the α-glucoside of *p*-hydroxybenzoic acid.

DURING a study of the influence of day length on the chemical composition of extracts of *Larix* needles, attention was drawn to a substantial amount of vanillic acid in acid hydrolysates. The existence of a glucoside of vanillic acid in several plants has been suggested,[1,2] but this compound has not been described. In this work a vanillic acid derivative (GV) was isolated (in solution) from needles of *Larix laricina* (Du Roi) K. Koch by repeated banding on chromatography paper. During this process two other glycosides, with rather similar R_f on paper, were found and similarly isolated. These compounds occur in the needles in similar amounts to that of GV.

With all three compounds, acid hydrolysis (N HCl for 0·5 hr at 100°) yielded a sugar residue and a phenolic acid. In all cases the sugar residue was identified as glucose by R_f (Table 1) and by its behaviour toward glucose oxidase. The acids were identified as vanillic acid, *p*-hydroxybenzoic acid and *p*-coumaric acid by R_f and spectral comparison (Tables 1 and 2) with authentic samples. For GV, the glucose/vanillic acid ratio was estimated from the intensity of the glucose spot on a silica HF thin-layer plate (developed with $CHCl_3$–HOAc–MeOH (45:20:35) and sprayed with *p*-anisidine/phosphoric acid) and from the u.v. absorbence of the acid, and appeared to be 1:1. Hydrolysis did not occur with any of the compounds under mild alkaline conditions (2 hr at room temperature in 2 N NaOH).[3] This provides evidence for glucosides, not glucose esters.

The occurrence of a free carboxyl group in the isolated compounds was indicated by the low R_f in IAmW (Table 1). More substantial evidence was obtained from the u.v. spectra (Table 2); the hypsochromic shift shown on addition of NaOAc indicates the presence of an acid group stronger than a normal phenolic hydroxyl. The same shift was obtained on the addition of NaOH, pointing to the absence of a free phenolic group which would give a bathochromic shift. Thus, GV is vanillic acid glucoside, and the other two compounds are

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[2] I. A. PEARL, D. L. BEYER and D. LASKOWSKI, *J. Org. Chem.* **24**, 443 (1959).
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TABLE 1. *Larix* PHENOLIC GLUCOSIDES. CHROMATOGRAPHIC RESULTS

Compound	Colour reactions*		R_f (x100)							
			Paper				Silica gel HF			
	DNA	DSA	BuAW†	2% HOAc	IAmW†	BzAW†‡	EMFW†	CAM 75†‡	CAM 45†	BzAM†
GV§	C	C	55	73	10		47	45		
GPH§	C	C	59	73	7		57	38		
GPC§	C	C	57	80	12		57	25		
Acid from GV	P	O	82	51	14	64				
Vanillic acid	P	O	84	51	14	64				
Acid from GPH	RR	Y	86	57	17	31				
p-Hydroxybenzoic acid	RR	Y	85	58	18	30				
Acid from GPC	B	R	84	31, 62	23, 30	45				
p-Coumaric acid	B	R	84	33, 62	24, 31	45				
Sugar from GV¶									62	82
Sugar from GPH¶									63	82
Sugar from GPC¶									61	82
Glucose¶									62	82

* DNA = 0·05% *p*-Nitrobenzenediazonium tetrafluoborate, DSA = diazotized sulfanilic acid, both oversprayed with 20% aqu. Na_2CO_3. C = colourless, P = purple, O = orange, RR = rose red, Y = yellow, B = blue, R = rose.

† BuAW = butanol–1/27% acetic acid (1:1), IAmW = isopropanol/ammonia/water (8:1:1), BzAW = benzene/acetic acid/water 6:7:3) upper layer, EMFW = ethyl acetate/methyl ethylketone/formic acid/water (5:3:1:1), CAM 75 = chloroform/acetic acid/methanol (75:5:20), CAM 45 = chloroform/acetic acid/methanol (45:20:35), BzAM = benzene/acetic acid/methanol (2:2:6). BzAM was used on plates impregnated with 0·1 N boric acid.

‡ R_f in these systems are not reproducible; average values are given. In CAM 75 a good separation was not always obtained.

§ GV = glucosylvanillic acid, GPH = glucosyl-*p*-hydroxybenzoic acid, GPC = glucosyl-*p*-coumaric acid.

¶ The sugars from GV, GPH, and GPC were also chromatographed on Whatman No. 1 paper with butanol-1/pyridine/water (10:3:3) with overdevelopment to distinguish between glucose and galactose. The three samples had the same R_f in this system as glucose.

TABLE 2. COLOURS AND U.V. SPECTRA OF *Larix* PHENOLIC GLUCOSIDES

Compound	Fluorescence colours*		Absorption maxima, λ_{max} in nm		
	At 254 nm	$+NH_3$	In EtOH	+NaOAc	+NaOH
GV†	VB	B	252, 290·5	244, 282, 286·5	245, 282, 286·5
Acid from GV	VB	B abs.	259, 291	248·5, 282, 288	283·5, 295
Vanillic acid	VB	B abs.	258·5, 290	250, 284,‡ 288	283, 296
GPH†	abs.	VB abs.	248	240	239·5
Acid from GPH	abs.	abs.	254	243·5	280
p-Hydroxybenzoic acid	abs.	abs.	254	245	280
GPC†	abs.	abs.	287	267	267
Acid from GPC	abs.	VB	227, 300,‡ 310	281, 301‡	309,‡ 335
p-Coumaric acid	abs.	VB	227·5, 300,‡ 310	284·5, 297‡	312,‡ 336

* VB = violet blue, B = blue, abs. = absorbent.

† GV = glucosylvanillic acid, GPH = glucosyl-*p*-hydroxybenzoic acid, GPC = glucosyl-*p*-coumaric acid.

‡ Shoulder.

p-coumaric acid glucoside (GPC) and *p*-hydroxybenzoic acid glucoside (GPH). Treatment with emulsin for half an hour at 37° hydrolysed GV and GPC but not GPH. This suggests an α-glucosidic link for GPH and a β-glucosidic nature for GV and GPC.

The β-glucoside of *p*-coumaric acid has been described[4, 5] but seems to have been isolated only from plants to which *p*-coumaric acid had been fed.[4] Strohl and Seikel[6] found 4-glucosyloxybenzoic acid in pine pollens, but no determination of the α- or β-glucosidic nature of the acid was made. Co-chromatography of Dr. Seikel's 4-glucosyloxybenzoic acid and *Larix* GPH in $CHCl_3$–HOAc–MeOH (75:5:20) on silica gel HF gave similar R_f.

[4] V. C. RUNECKLES and K. WOOLRICH, *Phytochem.* **2**, 1 (1963).
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EXPERIMENTAL*

Fresh needles (50 g) were frozen in liquid N_2, ground in a Waring blendor with a little ethanol, and extracted in the cold with 2 × 500 ml 70% ethanol during 48 hr. Successive banding with BuAW, with water or 2% HOAc, and with butanol-1/pyridine/water (14:3:3) of the concentrated extract on Schleicher and Schuell paper No. 2045B yielded a mixture of the three glycosides. GV was separated from the other two by banding on silica gel HF thin-layer plates with EMFW.[7] The plates were prewashed with a solvent mixture in which formic acid was replaced by acetic acid. Formic acid interferes with the fluorescent agent in the silica (Merck, HF); a total loss of the fluorescence of the plates resulted on repeated contact. The two remaining glycosides (GPH and GPC) were finally separated on paper with IAmW. The same system was used for a last purification of GV.

On paper the compounds were visible under u.v. light (λ 254 nm), but the fluorescence or absorbence was rather poor. The compounds could be detected by acid hydrolysis in N HCl for 0·5 hr at 100° and subsequent chromatography of the phenolic acids in BzAW. The phenolic acids were then detected by their fluorescence or absorbence under u.v. 254 nm and by a DNA spray. For detection of GV and GPC in mixtures generally a test strip was cut from the banded sheets and treated with emulsin in a moist chamber at 37°. The strip was then laid horizontally across the top of a clean sheet of paper between narrow glass plates, and the bands were developed in their second direction with BzAW. According to the u.v. absorbence, about 1–4 mg of each of the compounds were obtained from 50-g fresh needles.

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* See Table 1, footnotes † and * for the solvent systems and spray reagents used.

[7] K. RANDERATH, *Thin Layer Chromatography*, 2nd ed., p. 213, Verlag Chemie, Academic Press, New York (1966).