150. Structural Analysis of Eukovoside, A New Phenylpropanoid Glycoside from *Euphrasia rostkoviana* HAYNE[1])

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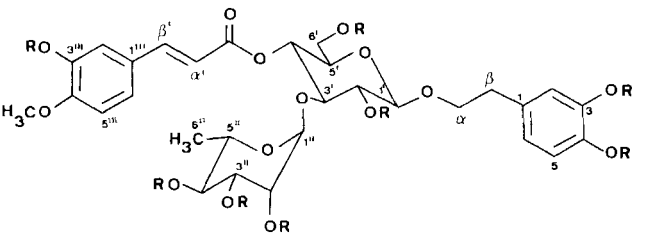
Summary

The isolation and structure determination of a phenylpropanoid glycoside from *Euphrasia rostkoviana* HAYNE, named eukovoside (**1**), are reported.

Introduction. – Our previous investigations on the glycosidic constituents of *Euphrasia rostkoviana* HAYNE resulted in the isolation and structure determination of several new iridoids [1] and a new lignan glucoside [2]. Fractionation of the water-soluble constituents on silica gel gave five fractions [2]. Further chromatography of fraction A on silica gel resulted in a new phenolic glycoside, named eukovoside for which spectral and chemical evidence led to structure **1**.

Results and discussion. – Eukovoside (**1**) was obtained as an amorphous powder, $[\alpha]_D^{20} = -93.6°$ ($c = 0.85$, CH_3OH) with the molecular formula $C_{30}H_{38}O_{15}$. Its UV. spectrum (CH_3OH) showed λ_{max} at 322 (4.33), 288 (4.18) *S* and 201 (4.54) nm.



1 Eukovoside R = H
2 Eukovoside octaacetate R = Ac

[1]) Part 3 in the series 'Glycosides of *Euphrasia* Species'. For part 2 see ref. [2].
[2]) Part of the Ph.D. thesis of *O. Salama*, ETH Zürich.

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The IR. spectrum (KBr) showed absorption for hydroxyl (3390 cm^{-1}, br.), conjugated ester (1700 cm^{-1}), C(α)=C(β) of isoferulic acid (1630 cm^{-1}) and the aromatic rings (1605, 1515 cm^{-1}). – ^{1}H- and ^{13}C-NMR. showed **1** to have aromatic and sugar moieties.

Acid hydrolysis of **1** in refluxing aqueous 2 N HCl/CH_3OH 1:1 afforded L-rhamnose, D-glucose and isoferulic acid. Milder hydrolysis gave L-rhamnose as the only detectable sugar, indicating it to be the terminal unit. The FD.-MS. data established the sequence to be rhamnose (terminal) – glucose – aglycone (m/z 492 = M^+ – thamnosyl and m/z 146 = rhamnosyl).

Acetylation of **1** under mild conditions provided the fully acetylated derivative **2**, $C_{46}H_{54}O_{23}$. The ^{1}H-NMR. spectrum of **2** revealed the presence of eight acetyl signals belonging to three aromatic – (δ 2.25–2.46) and five aliphatic (δ 1.82–2.10) acetyl groups.

The ^{1}H-NMR. spectra of **1** and its peracetate **2** *(Table 1)* suggested that the L-rhamnose unit is substituted only at the anomeric C-atom whereas the D-glucose unit is substituted in the 1, 3 and 4 positions. The configurations of the sugar linkage were deduced to be the β-D-form for glucose and the α-D-form for rhamnose by the ^{1}H-NMR. spectrum of **1** which exhibited the signals due to the anomeric protons of the glucose and rhamnose as doublets at δ 4.37 (J = 8 Hz), and δ 5.2 (J = 1.7 Hz), respectively. The ^{1}H-NMR. spectrum of **2** also supported this conclusion. The isoferuloyl group occupied the 4-position of the D-glucose, since H–C(4′) absorbs at low field in **1** (4.92 ppm) and is only slightly shifted upon peracetylation (5.22 ppm, signals assigned with the aid of extensive decoupling experiments).

Table 1. *^{1}H-NMR.* (360 MHz) *spectral data of eukovoside* (**1**) *and eukovoside octaacetate* (**2**)[a]

Proton(s) at	**1** (CD_3OD)	**2**[b]) ($CDCl_3$)
C(1′)	4.37 *d* (8.0)	4.39 *d* (8.0)
C(2′)	3.39 *d*×*d* (8.0/9.2)	5.09 *d*×*d* (8.0/8.0)
C(3′)	3.82 *t* (9.2)	3.90 *t*[c]) (8.5)
C(4′)	4.92 *t* (9.0)	5.22 *t* (9.0)
C(5′)	3.49–3.64	3.64 *m*
C(6′)		4.17 *m*
C(1″)	5.20 *d* (1.7)	4.85 *d* (1.7)
C(2″)		5.03 *m*
C(3″)	3.49–3.64	5.01–5.15 *m* (10.0/3.5)
C(4″)		4.96 *t* (10.0)
C(5″)		3.82 *m*[c])
C(6″)	1.10 *d* (7.5)	1.30 *d* (7.0)
C(α)	3.72/4.04 *m*	3.64/4.10 *m*
C(β)	2.79 *m*	2.86 *m*
C(α')	6.37 *d* (16.0)	6.35 *d* (16.0)
C(β')	7.65 *d* (16.0)	7.65 *d* (16.0)
C(4‴)–OMe	3.88 *s*	3.85 *s*
C(2/2‴)		
C(5/5‴)	6.54–7.20	7.00–7.15
C(6/6‴)		

[a]) Values in parenthesis are coupling constants in Hz. [b]) Additional signals: 1.82–2.10 (5×CH_3COO, aliphatic), 2.25–2.46 (3×CH_3COO, aromatic). [c]) Partly merged with the CH_3O–C(4‴) signal.

The ^{13}C-NMR. signals of **1** and **2** *(Table 2)* were assigned on the basis of chemical shift considerations, by single frequency-off-resonance decoupled data and comparison with data of corresponding esters [3], 3,4-dihydroxy-β-phenyl ethanol [4], glucose and rhamnose [5]. The substitution pattern on the glucose and rhamnose moieties was established in the following way, similar to that used for myricoside [6]: *a)* The oxygen functions at C(2′) and C(2″) are free hydroxyls since both the anomeric C-atoms are shifted upfield in the peracetate. This corroborates well with known upfield shifts accompanying acetylation of HO–C(2) due to a β-effect from neighbouring acyl groups [3]; *b)* the rhamnosyl and isoferuloyl moieties are attached to the glucose at C(3′) and C(4′), respectively, since the C(3′) signal undergoes an upfield shift (1.25 ppm) upon acetylation of HO–C(2′) as a result of the β-effect from the adjacent 2′-acetyl group. Additionally, in **1** and **2** the chemical shift values of the C(3′) signals agree well with the corresponding signals of acteoside and its peracetate [4b] as well as myricoside and its peracetate [6] which have similar substitution pattern on the glucose moiety; *c)* the 3,4-dihydroxy-β-phenylethanol moiety is attached to the glucose at C(1′) as evident from the chemical shift value (104.00 ppm) of that C-atom. In fact, it has been observed [7] [8] that the anomeric C-atom signal is deshielded in the decreasing order of primary, secondary and tertiary alcoholic-β-D-glucopyranosides.

The structure of eukovoside is thus deduced to be 3,4-dihydroxy-β-phenylethoxy-α-L-rhamnopyranosyl-(1→3)-4-*O*-isoferuloyl-β-D-glucopyranoside (**1**). Eukovoside is related to a few similar phenylpropanoid glycosides with two (acteoside [9], isoacteoside [6], conandroside [10], martynoside [11], forsythoside A [12]) or three sugar moieties (echinacoside [13], neoacteoside [9], magnolidin [14], myricoside [6]). Of these, echinacoside, forsythoside A and myricoside are of biological interest. The former two showed potent antibacterial and the last one potent antifeedant

Table 2. *^{13}C-NMR. spectral data of eukovoside* (**1**) *and eukovoside octaacetate* (**2**)[a])

C-Atom	1	2	C-Atom	1	2
C(1)	131.44 *s*	137.58	C(1″)	102.85 *d*	98.97
C(2)	116.37 *d*	123.85	C(2″)	72.16 *d*	70.69
C(3)	144.45 *s*	141.86	C(3″)	72.16 *d*	68.61
C(4)	145.90 *s*	141.86	C(4″)	73.70 *d*	72.05
C(5)	117.14 *d*	123.14	C(5″)	70.31 *d*	67.26
C(6)	121.14 *d*	123.43	C(6″)	18.41 *qa*	17.49
C(1′)	104.00 *d*	100.69	C(1‴)	127.53 *s*	132.94
C(2′)	75.98 *d*	72.22	C(2‴)	115.80 *d*	121.34
C(3′)	81.53 *d*	80.28	C(3‴)	149.20 *s*	140.57
C(4′)	70.47 *d*	69.54	C(4‴)	150.58 *s*	151.55
C(5′)	75.75 *d*	70.07	C(5‴)	111.80 *d*	111.47
C(6′)	62.25 *t*	62.32	C(6‴)	124.26 *d*	127.26
OCH_3	56.46 *qa*	55.34			
C(α)	72.16 *t*	69.73	C(α')	115.00 *d*	117.06
C(β)	36.40 *t*	35.36	C(β')	147.92 *d*	145.68
CO	168.32 *s*	165.19–170.70	CH_3(Ac)	–	20.59–21.05

[a]) The spectra were recorded in CD_3OD (**1**) and $CDCl_3$ (**2**). Chemical shift in ppm relative to internal TMS.

activities. Eukovoside showed no antifeedant activity (tested against *Spodoptera littoralis, Anthonomus grandis* and *Diabrotica balteata*)[3]).

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Experimental Part

General. Melting points were determined on a *Mettler Fp5/FP52* apparatus. UV. spectra [λ_{max} ($\log\varepsilon$)] were determined in CH_3OH for spectroscopy *(Merck)* on a *Perkin-Elmer 550* spectrometer. IR. spectra (cm^{-1}) were determined on a *Perkin-Elmer 257* instrument in KBr pellets. ^1H- and ^{13}C-NMR. spectra (δ in ppm, J in Hz) were obtained at 360 MHz using a *Bruker Spectrospin* instrument and at 25.2 MHz in *Fourier* transform mode using a *Varian XL-100-12* spectrometer, respectively, with tetramethylsilane as an internal standard. EI.-MS. (*m/z*) were recorded with a *Hitachi-Perkin-Elmer RMU 6M* spectrometer and FD.-MS. with a *Varian CH 5* spectrometer. Silica gel 60 (70–230 mesh, *Merck*) and neutral alumina (*Woelm* N, Act. 1) were used for column chromatography and silica gel *60 F_{254} (Merck)* prepared plates for TLC. Spots were detected by UV. fluorescence and spraying with vanillin/H_2SO_4 followed by heating at 100° for 5–10 min. For HPLC. detection a *Waters* Assoc. *M-6000* pump as the solvent delivery system, a U6K septumless injector and a *μ-Bondapak* C_{18} column (30 cm × 3.9 mm I.D.) were used. The system was equipped with a *Zeiss* spectrophotometer (*PMQ3* with microcell MR 1D 1 cm) with a variable wave length detector and a W+W recorder series 1100. Abbreviations: HPLC. = high performance liquid chromatography, TLC. = thin layer chromatography, PIC(TM) = tetrabutylammonium phosphate (*Waters* Assoc., Inc.).

Extraction. Dried and milled whole plant (1 kg) of *Euphrasia rostkoviana*, available commercially from *Siegfried AG* (Lot No. 19279), Zofingen, Switzerland, was extracted with CH_3OH at 40° (4× 5 l). After concentration of the combined extracts *in vacuo*, H_2O (1.5 l) was added and the H_2O-insoluble material removed by filtration through *Celite*. The filtrate was extracted with petroleum ether (60–80°, 4× 1 l) and the soluble part was rejected. The aqueous layer was concentrated (200 ml) and filtered through a prewashed (H_2O) neutral Al_2O_3 (500 g) column eluting with H_2O. The aqueous eluate was concentrated and lyophilized to give the crude glycoside fraction (45 g). A portion of the residue (25 g) was chromatographed over silica gel (400 g) with $CH_2Cl_2/CH_3OH/H_2O$ 4:1:0.1 (3 l), 7:3:0.3 (3 l), 1.4:1:0.1 (2 l) and 5 fractions A–E were collected.

Isolation of eukovoside (**1**). Fraction A (1.2 g) was chromatographed over silica gel (72 g) with AcOEt/PrOH/H_2O [4:2:7, upper layer] and two fractions A_1 (0.4 g) and A_2 (0.37 g) were collected. Fraction A_1, further chromatographed over silica gel eluting with $CH_2Cl_2/CH_3OH/H_2O$ 4:1:0.1, gave pure **1** (145 mg), $[\alpha]_D^{20}$, UV., IR.: see text. – ^1H-NMR. and ^{13}C-NMR. (*Tables 1* and *2*). – FD.-MS.: 639 (100, $(M+H)^+$), 503 (85, $(M-135)^+$), 492 (7, $(M-146)^+$), 462 (85, $(M+H)-177)^+$), 146 (14), 136 (57, $(135+H)^+$).

Total hydrolysis of eukovoside (**1**). Acid hydrolysis of **1** (15 mg) in 2 ml refluxing aqueous 2N HCl/CH_3OH 1:1) for 2 h yielded L-rhamnose, D-glucose and isoferulic acid. The sugars were detected by TLC. (silica gel, BuOH/AcOH/H_2O 4:1:2) and isoferulic acid by HPLC. (reversed phase, CH_3OH/H_2O 3:7 mixed with PIC(TM)).

Partial hydrolysis of eukovoside (**1**). Mild hydrolysis of **1** (10 mg) in 2 ml refluxing aqueous 0.1N HCl for 30 min gave L-rhamnose as the only detectable sugar.

Eukovoside octaacetate (**2**). Acetylation of **1** (50 mg) with acetic anhydride/pyridine at RT. for 2 h followed by column chromatography over silica gel using AcOEt/ether 1:1 gave eukovoside octaacetate (**2**). The product was recrystallized from ethanol to give **2** (68 mg) as fine needles, m.p. 124–126°, $[\alpha]_D^{20} = -52.1°$ ($c = 0.61$, $CHCl_3$). – UV. (MeOH): 313 (4.01) *S*, 282 (4.29), 202 (4.50). – IR. (KBr): 1700 (C=O, ester), 1635 (C=C), 1600 and 1510 (arom. ring). – ^1H-NMR. and ^{13}C-NMR. (*Tables 1* and *2*). – FD.-MS.: 998 (61, $(M+Na+H)^+$), 997 (22, $(M+Na)^+$), 975 (15, $(M+H)^+$), 974 (61, $(M)^+$), 932

[3]) The authors wish to thank Prof. *K. Nakanishi*, Columbia University, New York, and Dr. *Th. Schläpfer*, Ciba-Geigy AG, Basel, for carrying out the leaf disk bioassays.

(100, $(M+H-CH_3COO)^+$). – EI.-MS.: 974 (M^+, no peak), 932 (1), 890 (2), 848 (6), 737 (3), 714 (3), 695 (2), 672 (1), 643 (1), 601 (1.5), 561 (2), 558 (2), 493 (1.7), 470 (1), 465 (1.5), 451 (4), 443 (2), 423 (11), 407 (1.5), 377 (1), 331 (1), 317 (4), 289 (1), 273 (100), 247 (1), 236 (1), 231 (20), 219 (7), 213 (29), 194 (14), 189 (6), 177 (77), 171 (10), 153 (71), 145 (17), 137 (55), 127 (10), 123 (8), 119 (7), 111 (70), 103 (2), 99 (6), 83 (26), 69 (6), 60 (3), 47 (70).

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