Phytochemistry, Vol 27, No 5, pp 1459–1463, 1988
Printed in Great Britain.

0031–9422/88 $3.00 + 8.00
Pergamon Press plc

TEUCRIOSIDE, A PHENYLPROPANOID GLYCOSIDE FROM *TEUCRIUM CHAMAEDRYS*

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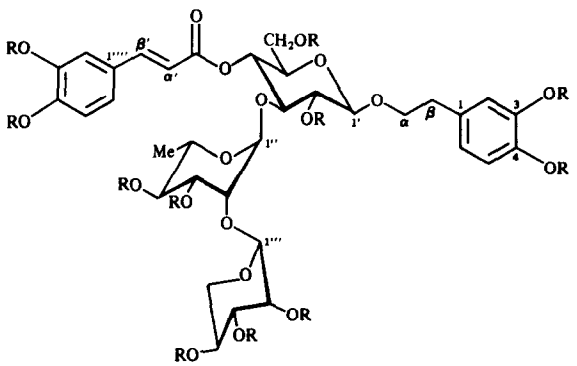
(*Received* 24 *August* 1987)

Key Word Index—*Teucrium chamaedrys*; Labiatae; phenylpropanoid glycosides, teucrioside; acteoside (verbascoside); caffeic acid; L-lyxose; FDMS; ^{1}H and ^{13}C NMR

Abstract—The structure of teucrioside, a new phenylpropanoid glycoside isolated from *Teucrium chamaedrys* has been elucidated as 3,4-dihydroxy-β-phenylethoxy-*O*-α-L-lyxopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-4-*O*-caffeoyl-β-D-glucopyranoside.

INTRODUCTION

Different species of the genus *Teucrium* are used in folk medicine and have been extensively investigated for their chemical constituents (e.g. diterpenes, monoterpenes, as well as glycosides such as iridoids, saponins and flavonoids). Petričić *et al* [1] reported the isolation of three glycosides from *Herba teucrii montani* which afforded two to four different sugars upon acid hydrolysis. Since the aglycone part of these compounds and their substitution patterns were not established, we became interested in investigating the glycosidic constituents of various *Teucrium* [2] species This paper reports the structure determination of a new phenylpropanoid glycoside (**1**) from *Teucrium chamaedrys* L. By using 1- and 2D NMR experiments, all assignments of carbon-13 signals of the sugar moieties could be established with accuracy on the underivatized substance.



1 R = H
2 R = Ac

RESULTS AND DISCUSSION

Plant material from the commercially available wall germander (*Herba chamaedryos*) was extracted with methanol. The water-soluble part of the methanol extract was concentrated, lyophilized, and fractionated over polyamide and cellulose columns resulting in pure, amorphous teucrioside (**1**), $C_{34}H_{44}O_{19}$ (FDMS). It exhibited UV absorptions at 203, 288, and 332 nm which were shifted by sodium methoxide to 255, 310, and 375 nm, confirming its polyphenolic nature. IR bands for hydroxyl groups (3400 cm^{-1}, *br*), an α,β-unsaturated ester ($\nu_{C=O}$ 1700 cm^{-1}, $\nu_{C=C}$ 1635 cm^{-1}), and aromatic rings (1610, 1520 cm^{-1}) were also observed.

Acid hydrolysis of **1** in refluxing 2 M HCl–MeOH (1:1) yielded caffeic acid as well as three sugar moieties which were detected by TLC, two of which could be unambiguously identified as rhamnose and glucose. GC analysis of the aldonitrile acetate derivatives, however, suggested the identity of the third sugar to be lyxose. After hydrolysis of **1** in aqueous 0.2 M HCl followed by chromatographic work-up of the aqueous phase on Sephadex LH-20, acetoside (TLC, HPLC, $[\alpha]_D$) and lyxose (TLC) were identified [despite the identical R_f-values of xylose and lyxose, they can be distinguished by their colour reaction after heating the chromatoplate with the spray reagent (see Experimental)]; hence, lyxose had to be in the terminal position. Hydrolysis of **1** with 2 M trifluoroacetic acid yielded rhamnose and lyxose. After acetylation, this mixture was chromatographically separated into the two sugar acetates. Comparing the ^{1}H NMR spectra of an authentic sample of lyxose tetraacetate with that of the isolated compound confirmed its identity (see Table 1). In view of the NMR spectroscopically proved α-glycosidical junction between lyxose and rhamnose (see below), it can be concluded that the lyxose moiety in **1** has L-configuration, since the difference of molecular rotations between **1** and acteoside [3] indicates a negative rotatory contribution of the lyxose part in **1**. $[M]_1-[M]_{acteoside} = -47.3°$; $[M]_{\alpha\text{-L-lyxose peracetate}} = -58.1°$; $[M]_{\alpha\text{-D-lyxose peracetate}} = +63.7°$ (for further details see Experimental).

FDMS of **1** (Fig. 1) gave the $[M+Na]^+$ ion at *m/z* 779 as the base peak. In addition, the $[M+H]^+$ ion at *m/z*

Table 1 ^{1}H NMR spectral data of compounds **1** and **2***

H	**1** (CD_3OD, 500 MHz)	**2**‡ ($CDCl_3$, 300 MHz)
Aglycone		
2	6 708 *d* (2 2)	7 03–7 05 *m*
5	6 687 *d* (8 7)	7 03–7 05 *m*
6	6 561 *dd* (2 2/8 7)	7 03–7 05 *m*
α	4 03 *m*	4 12† *m*
	3 71†	3 6–3 87† *m*
β	2 79 *m* (2H)	2 87 *m*
Glucose		
1′	4 263 *d* (7 8)	4 4 *d* (8)
2′	3 389 *dd* (7 8/9)	5 05†
3′	3 780 *t* (9)	3 98 *t* (8)
4′	4 900†	5 21 *t* (8)
5′	3 520†	3 60–3 87†
$6'_A$	3 54†	4 17–4 19†
$6'_B$	3 64†	4 17–4 19†
Rhamnose		
1″	5 390 *d* (1 6)	4 95 *br s*
2″	3 959 *dd* (1 6/3 3)	3 90 *m*
3″	3 688 *dd* (3 3/10)	4 88–4 98†
4″	3 290 *t* (10)	4 88–4 98†
5″	3 540 *m*	3 68–3 72†
6″	1.071 *d* (6 3)	1 07 *d* (6 2)
Lyxose		
1‴	4 900 *d* (3 3)	4 7 *d* (3 6)
2‴	3 874 *t* (3 3)	5 2 *t* (3 6)
3‴	3 725†	5 34 *dd* (8 1/3 6)
4‴	3 757†	5 00–5 11†
5‴	3 580†	3 60–3 87†
5‴	3 700†	3 60–3 87†
Caffeic acid		
2⁗	7 065 *d* (2 2)	7 35 *d* (1 9)
5⁗	6 786 *d* (8 7)	7 22 *d* (8 3)
6⁗	6 948 *dd* (2 2/8 7)	7 38 *dd* (1 9/8 3)
α′	6 28 *d* (15 35)	6 35 *d* (16)
β′	7 6 *d* (15 35)	7 69 *d* (16)

*Chemical shifts in ppm relative to internal TMS
Values in parenthesis are coupling constants in Hz
†Signal pattern unclear due to overlapping
‡Teucrioside undecaacetate has additional signals 2 3, 2 29, 2 28, 2 27 (4 × MeCOO, arom.), 2 1, 2 08, 2 05, 2 03, 2 01, 1 94, 1 74 (7 × MeCOO, aliph.)

757 and the $[M+{}^{39}K]^+$ ion at *m/z* 795 were found From these signals and the doubly charged ion at *m/z* 401 for $[M+2\,Na]^{2+}$, the M_r of **1** was clearly determined The structural features were revealed firstly by the $[(M+Na)-132]^+$ ion at *m/z* 647 for the loss of the lyxose moiety and by the $[(M+Na)-278]^+$ ion at *m/z* 501 for the loss of the diglycosidic chain (Rha-Lyx), thereby confirming the linkage of lyxose on the rhamnose moiety Secondly, the prominent ion at *m/z* 617 indicated cleavage of the caffeoyl group. This assignment was supported by the two signals at *m/z* 485 and 339 for $[(M+Na)-162-132]^+$ and $[(M+Na)-162-278]^+$, respectively Furthermore, this favoured fission between the carbonyl function of the caffeoyl rest and the glycosidic oxygen gave a prominent doubly charged ion at *m/z* 320 for the $[(M+2\,Na)-162]^{2+}$ signal

Acetylation of **1** gave the undecaacetate **2** The FDMS of **2** (Fig. 2) showed the $[M+Na]^+$ ion as the base peak at *m/z* 1241, an $[M+H]^+$ ion at *m/z* 1219, and a doubly charged $[M+2\,Na]^{2+}$ ion at *m/z* 632, all of which correspond to the molecular formula $C_{56}H_{66}O_{30}$ As shown for peracetylated phlorotannins [4] with FDMS at higher emitter heating currents, thermal elimination of ketene in the adsorbed sample on the emitter surface can be induced Starting from the molecular cluster, a series of four ketene eliminations is registered at *m/z* 1199, 1157, 1115 and 1073 Similarly, as for **1**, loss of the caffeoyl moiety yields an intense fragment Thus at *m/z* 1037, the $[(M+Na)-204]^+$ ion is recorded with 18% relative abundance and is also accompanied by a series of three ketene losses indicated at *m/z* 995, 953, 911 When using these soft ionization conditions, intense ions in the lower mass range are not observed

The ^{1}H NMR spectrum of **1** closely resembled that of acteoside. six aromatic protons (2 × ABX, δ6 56 – 7 07), the coupling constants of which gave the substitution pattern indicated in the formula, two *trans* olefinic protons [AB, δ6 28, 7 6 (2 × 1 H, J = 15 35 Hz)], and two methylene groups (H_2-β, *t*, *d* 2 79, H_2-α, 2 × *m*, δ3 71, 4 03) Two doublets at δ4 26 (J = 7 8 Hz) and at 5.39 (J = 1 6 Hz) were consistent with the configuration β for D-glucose and α for L-rhamnose The 3 3 Hz coupling constant of the anomeric H (δ4 9) of lyxose indicated its α-configuration This assignment was confirmed by comparing $J_{1,2}$ of **1** and **2** respectively with that of authentic lyxose (measured at 300 13 MHz in CD_3OD) which occurs in solution as a 7 3 mixture of the α-($J_{1,2}$ = 3 74 Hz), and β-($J_{1,2}$ = 2 2 Hz) forms [5] The chemical shifts of the strongly overlapping ring proton signals (see Table 1) of the three sugar moieties were confirmed by a homonuclear correlated 2D NMR spectrum of **1** at 500 MHz The ^{1}H NMR spectrum of the undecaacetate **2** (1D, 300 MHz) showed the presence of four aromatic and seven aliphatic acetyl groups No downfield shift upon acetylation was observed for H-2 of the rhamnose which, therefore, had to be substituted at hydroxyl C-2 The signals of H-1, H-2, and H-3 of the lyxose moiety were well separated from the remaining signals and confirmed the already established configuration All assignments were confirmed by selective decoupling experiments.

The ^{13}C NMR signals of **1** (see Table 2) were assigned with the help of two C,H-heteronuclear correlated 2D NMR spectra; one for establishing direct C–H connectivities and another for long-range coupling relations This way a discrepancy in the literature concerning the assignment of the caffeate moiety singlets of C-3 (δ_C = 146 18) and C-4 (δ_C = 146 98) could be clarified Some authors [3, 6, 7] assign these carbon signals in a reversed way Our experiment, however, shows that the assignment given in Table 2 is correct The data from related compounds such as acteoside [8, 9], eukovoside [10]* or

*A detailed ^{1}H NMR analysis revealed the ester moiety in eukovoside to be feruloyl not isoferuloyl as we inadvertently reported earlier [10] Alkaline hydrolysis of eukovoside and characterization of the acid by HPLC, TLC and ^{1}H NMR showed it to be ferulic acid Thus, the structure of eukovoside should be written as 3,4-dihydroxy-β-phenylethoxy-α-L-rhamnopyranosyl-(1→3)-4-*O*-feruloyl-β-D-glucopyranoside, which is identical to that proposed for leucosceptoside A [11] (for direct comparison of authentic compounds, see Experimental)

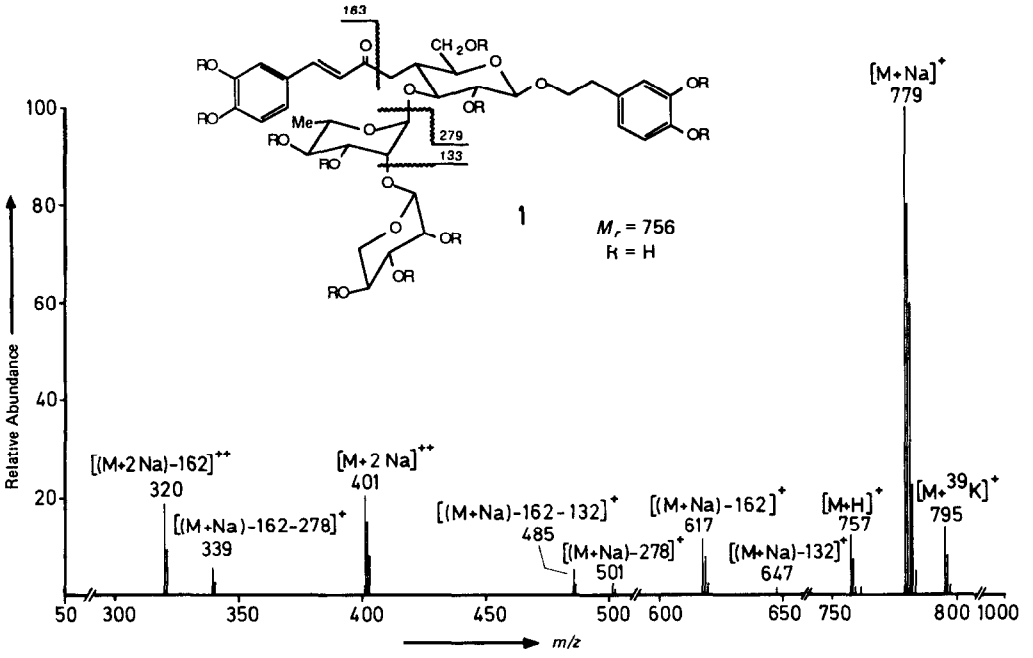


Fig 1. FDMS of **1**. Methanol was used as solvent The ion source potentials were +8 kV for the high temperature emitter and −4 kV for the counter electrode Electric registration and accumulation of 20 spectra between 25 and 35 mA emitter heating current with the Finnigan SS20 data system was performed

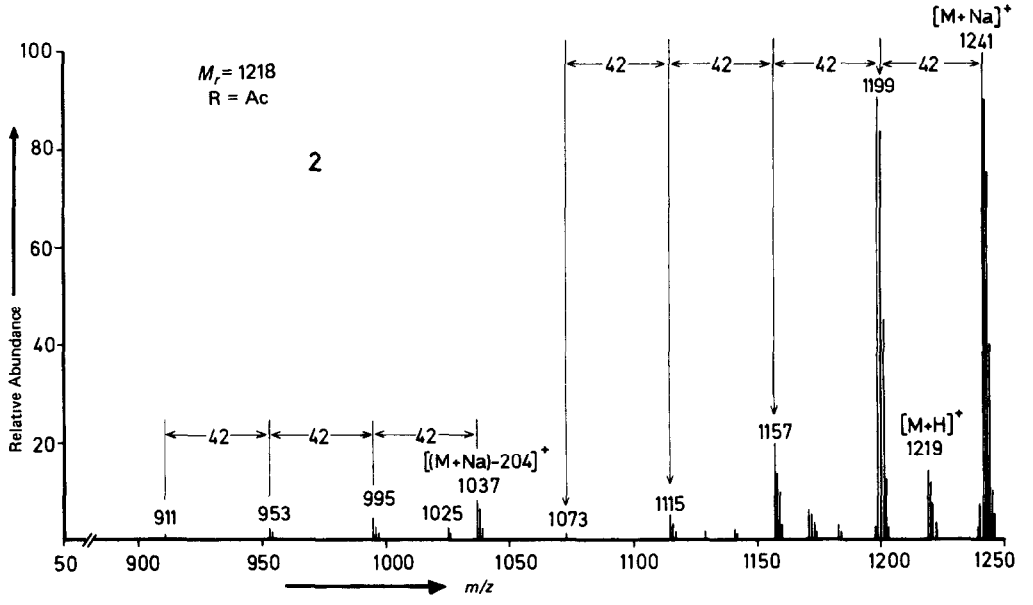


Fig 2 FDMS of **2** Same experimental conditons as described for **1**, except with a lower range of emitter heating currents (20–30 mA)

myricoside [12] served as comparisons. Thus the signals of structural parts common in acteoside and **1** were virtually superimposable. The main problem, the linkage position of the lyxose unit to the rhamnose, as expected, could be demonstrated to be the hydroxyl at C-2″, since in the 13C NMR spectrum the C-2″ signal is located at 80.66 ppm in **1**. This assignment is correct thanks to the 2D correlation to H-2″. Thus, the structure of teucrioside (**1**) was confirmed to be 3,4-dihydroxy-β-phenylethoxy-*O*-α-L-lyxopyranosyl(1→2)-α-L-rhamnopyranosyl-(1→3)-4-*O*-caffeoyl-β-D-glucopyranoside. The formerly proposed structure of teucrioside [2] has, therefore, to be corrected Although the structure of teucrioside is very similar to that of myricoside, which showed

Table 2. ^{13}C NMR spectral data of compound **1** (125.47 MHz, CD_3OD, TMS as int. standard)

C	Aglycone		Caffeic acid
1	131.76 *s*	1	127.80 *s*
2	117.31 *d*	2	115.49 *d*
3	145.90 *s*	3	146.18 *s*
4	144.43 *s*	4	146.98 *s*
5	116.39 *d*	5	116.51 *d*
6	121.37 *d*	6	127.23 *d*
α	72.16 *t*	α′	123.57 *d*
β	36.39 *t*	β′	148.27 *d*
		CO	168.36 *s*

	Glucose		Rhamnose		Lyxose
1′	104.40 *d*	1″	102.14 *d*	1‴	104.40 *d*
2′	76.37 *d*	2″	80.66 *d*	2‴	71.62 *d*
3′	80.66 *d*	3″	72.20 *d*	3‴	72.83 *d*
4′	70.77 *d*	4″	74.40 *d*	4‴	69.12 *d*
5′	76.10 *d*	5″	70.77 *d*	5‴	65.11 *t*
6′	62.50 *t*	6″	18.85 *q*		

antifeedant activity, no such biological activity could be found when tested against *Spodoptera littoralis*, *Anthonomus grandis* and *Diabrotica balteata*. It may be possible that the furanoid ring structure occurring in myricoside (apiose unit) is of significance for this kind of biological activity. Teucrioside is the first L-lyxose containing phenylpropanoid glycoside found in nature. L-Lyxose has been reported to occur in yeast ribonucleic acid [13] and in the antibiotic curamycin-1[14]. As a secondary product in higher plants, lyxose is only known to occur glycosidically linked to the triterpene barringtogenol in *Planchonia careya* [15] (the authors [15] do not report to which configurational series (D or L) the lyxose moiety in the isolated substance belongs).

EXPERIMENTAL

General procedures. Mps uncorr. ^1H and ^{13}C NMR spectra (δ ppm, J Hz) were obtained at 300.13 and 500.13 MHz (^1H NMR) and at 75.47 and 125.47 MHz (^{13}C NMR) in FT mode using Bruker WM 300 (1D) and AM 500 (2D) instruments with TMS as an int. standard. For the ^1H–^1H-correlated spectrum (COSY), 1024 FID's were acquired (16 for each value of t_1) multiplied by a sinebell function to give an initial data matrix ($t_1 \times t_2$) of 1024 × 1024 points that was zero filled to 2048 × 2048 points, and then doubly transformed to give a matrix (real part, $f_1 \times f_2$) of 1024 × 1024 points for which the spectral width was 3521 Hz (7.04 ppm) and the digital resolution 3.44 Hz/point (=0.0068 ppm/point). In the ^1H–^{13}C-correlated spectrum, the following parameters were used: 512 FID's, initial data matrix 512 × 1024 points, zero filling to 1024 × 2048 points, real part of the transformed matrix 512 × 1024 points, spectral width 15481 Hz (f_1, 15.11 Hz/point), 3516 Hz (f_2, 6.86 Hz/point). The 2D spectra were evaluated as contour plots. EIMS 70 eV, *m/z*. A description of the Experimental conditions of FDMS has been given in refs [16, 17]. LPLC was carried out on a FPLC system Labochrom® (Labomatic) columns (713 mm × 18 mm i.d.) filled with Sepralyte C18 (40 μm, Analytichem), UV detection 206 nm. Polyamide (Woelm) and cellulose (Macherey, Nagel & Co.) were used for open CC, and silica gel 60 F_{254} (Merck) prepared plates for TLC. Spots were detected by UV fluorescence and spraying with vanillin–H_2SO_4 or naphthoresorcine–H_2SO_4 reagent (for sugars) followed by heating at 100° (Camag TLC plate heater II) for 5 min. Analytical HPLC: Knauer Lichrosorb RP18 (25 cm × 4 mm i.d.) (A), and Spherisorb ODS II (10 cm × 4 mm i.d.) (B) columns were used. The system was equipped with a Hewlett-Packard 1040 A high-speed spectrophotometric detector. Self-filled cartridges [Dowex 1X4, alumina (neutral (Woelm)] or silica gel (40–63 μm (Merck)), dimensions 1.2 × 5 cm or 0.8 × 3.5 cm) were used for sample clean-up prior to the HPLC/GC/TLC analysis. FID-GC: columns (2 m × 0.2 mm) filled with 0.2% PEGS, PEGA, XE60, and SE30 each on Gas-Chrom Q. Authentic D- and L-lyxose were obtained from Fluka (Switzerland).

Extraction and prepurification. 1 kg dried and milled plant material (*Teucrium chamaedrys* L., whole plant, commercially available from Dixa AG (Lot No. 679), St. Gallen, Switzerland) was extracted with MeOH at 40° (3 × 5 l). The combined extracts were evapd. under vacuum nearly to dryness, H_2O (1 l) was added and the H_2O-insoluble material removed by filtration through celite. The filtrate was extracted with petrol (60–80°, 3 × 1 l), and the soluble part was rejected. The aq. part was concentrated to about 200 ml and lyophilized to give a crude extract (142 g). A part of the residue (14.2 g) was chromatographed over polyamide (100 g) eluting with H_2O, followed by increasing concentrations of MeOH, and four fractions A (H_2O), B (10% MeOH), C (25% MeOH), D (50% MeOH) were collected.

Isolation of teucrioside (**1**). Fraction B (1.1 g) was chromatographed over cellulose (100 g) eluting with EtOAc–MeOH–H_2O (100:16.5:13.5) to give pure (HPLC) **1** (0.6 g), $[\alpha]_D^{25} = -76.0°$ ($c = 0.91$, MeOH). UV λ_{max}^{MeOH} nm: 203 (4.56), 288 (*sh*, 4.08), 332 (4.23), shifted upon addition of NaOMe to 255, 310 and 375. IR ν_{max}^{KBr} cm^{-1}: 3400 (*br*, OH), 1700 (C=O), 1635 (C=C), 1610, 1520 (arom. rings). ^1H and ^{13}C NMR (CD_3OD): see Tables 1 and 2. FDMS: see text and Fig. 1.

Total hydrolysis of teucrioside (**1**). Acid hydrolysis of **1** (50 mg) in 2 ml refluxing aq. 2 M HCl–MeOH (1:1) for 2 hr yielded lyxose, rhamnose, glucose, and caffeic acid. The Et_2O extract was used for HPLC detection of caffeic acid [column (A), MeOH–H_2O–AcOH, 30:70:0.5]. The aq. phase was diluted with H_2O and neutralized and purified using anion exchange resin and alumina cartridges. The eluate was evapd. under vacuum. The residue was dissolved in H_2O–MeOH (1:1) and used for detection of the sugars by TLC (silica gel, *iso*PrOH–EtOAc–H_2O (7:2:1), lyxose R_f 0.7, rhamnose R_f 0.77, glucose R_f 0.53). Although lyxose and xylose showed the same R_f value on TLC in all solvent systems tested, they were distinguishable by the colouration after heating the sprayed plates. After 1 min at 100°, lyxose gives a grey spot, while the xylose spot colour is blue. After several minutes, they become indistinguishable, exhibiting the same stable blue colouration.

Partial hydrolysis of teucrioside (**1**). Mild acid hydrolysis of **1** (100 mg) in 2 ml refluxing aq. 0.2 M HCl for 60 min gave lyxose as the only detectable sugar. The aq. phase was neutralized and lyophilized. Chromatography on Sephadex LH-20 (2.5 × 80 cm, mobile phase MeOH–H_2O, 1.9, 1 ml/min, fraction size 10 ml, UV detection 254 nm) afforded, in equal parts, unhydrolysed **1** as well as a second compound which was shown to be identical with aceteoside (HPLC, column A, mobile phase H_2O–THF–phosphoric acid, 80:20:0.25, 1.5 ml/min, detection at 320 nm, $[\alpha]_D^{20} = -78.7°$ ($c = 0.69$, MeOH).

Hydrolysis of **1** (60 mg) in refluxing 2 M CF_3COOH for 60 min and filtration of the neutral aq. phase through an alumina cartridge afforded a crude mixture consisting mainly of lyxose, rhamnose, and trace amounts of glucose. Acetylation of this mixture was carried out in the usual way (C_5H_5N–Ac_2O 1:1,

12 hr room temp.), TLC $CHCl_3$–MeOH 99 5 0 5, R_f 0 56 (lyxose), R_f 0.25 (rhamnose). Separation of the acetylated mixture (24 mg) was achieved by CC (stat phase silica gel (1 g), mobile phase: $CHCl_3$, 12 ml/hr, fractions of 1 ml were collected, lyxose acetate eluted in fractions 4–6 (11 mg) $[\alpha]_D^{20} = -20\,4°$ ($c = 1\,12$, $CHCl_3$) ^{1}H NMR see Table 1

Teucrioside undecaacetate (**2**). Acetylation of **1** (100 mg) with Ac_2O/C_5H_5N at room temp for 4 hr followed by CC over silica gel using $CHCl_3$–benzene–MeOH (3 1 0 1) gave **2**, which was crystallized from EtOH (130 mg), mp 94–95°, $[\alpha]_D^{25} = -62\,9°$ ($c = 0\,56$, $CHCl_3$) UV λ_{max}^{MeOH} nm· 206 (4 54), 282 (4 35) IR ν_{max}^{KBr} cm^{-1} 1750 (C=O), 1640 (C=C), 1505 (arom ring) ^{1}H NMR ($CDCl_3$) see Table 1 FDMS see Fig 2 EIMS 778 (2 2), 736 (0.7), 489 (31.4), 331 (5 2), 273 (3 9), 260 (8 3), 259 (63 7), 247 (3.3), 231 (3 9), 221 (2 3), 219 (2 1), 217 (6 6), 207 (1.7), 205 (6 2), 199 (22 9), 193 (2.5), 189 (2 3), 179 (8 4), 177 (5 2), 169 (39 6), 165 (2.4), 163 (11 6), 162 (13.9), 157 (35), 153 (5 3), 151 (2.8), 139 (33 5), 137 (17.6), 135 (2 7), 123 (4 1), 115 (8 2), 111 (9.3), 109 (25), 107 (3), 105 (4.2), 103 (5), 100 (14 5), 97 (91 3), 93 (1 9), 91 (4 3), 85 (21.7), 81 (9), 79 (2 5), 77 (3.3), 69 (7 4), 60 (6 3), 57 (5.5), 55 (5 9), 45 (7), 44 (5 5), 43 (100), 42 (71), 41 (16.6), 29 (5), 28 (14 2), 15 (6 1), 14 (19 6) Peaks with an intensity below 5% are only listed if they are of significance for the fragmentation pattern

Aldonitrile acetates 0 4 ml C_5H_5N and 10 mg hydroxylamine HCl were added to about 2 mg of sample of the above described hydrolysate (CF_3COOH). The mixture was kept at 90° for 30 min and cooled. After addition of 1 5 ml Ac_2O and subsequent heating for another 30 min, the solvent was evaporated *in vacuo* and the residue dissolved in 0 4 ml $CHCl_3$. 2 μl were injected into the GC temp program 160°–200° (3°/min), injector and detector temp. 225° both, R_ts (min, AA = aldonitrile acetate). 8 83 (rhamnose AA), 11 21 (lyxose AA) Pure lyxose AA: 11 22. Standard sample mixture. 8 82 (rhamnose AA), 10 42 (fucose AA), 11.27 (lyxose AA), 11 72 (arabinose AA), 13 0 (xylose AA), 14 62 (xylitol AA (int standard)), 17 95 (mannose AA), 20 27 (glucose AA), 21 7 (galactose AA).

Lyxose tetraacetate About 50 mg each of D-and L-lyxose were acetylated in the usual way (C_5H_5N–Ac_2O 1 1, 12 hr room temp.) and purified through silica gel cartridges ($CHCl_3$–MeOH, 199·1). L-lyxose tetraacetate was analysed by ^{1}H NMR (300 13 MHz, CD_3OD)· α-L-lyxose tetraacetate (≅70%) δ5.99 (1H, *d*, $J_{1,2} = 3.4$ Hz, H-1), δ5.24 (1H, *t*, $J_{2,1} = J_{2\,3} = 3\,4$ Hz, H-2), δ5.36 (1H, *dd*, $J_{3,2} = 3\,4$ Hz, $J_{3,4} = 8\,8$ Hz, H-3), δ5 19 (1H, *td*, $J_{4,3} = J_{4,5\text{-ax}} = 8\,8$ Hz, $J_{4,5\text{-eq}} = 4.9$ Hz, H-4), δ3.65 (1H, *dd*, $J_{5\text{-ax},5\text{-eq}} = 11.5$ Hz, $J_{5\text{-ax},4} = 8.8$ Hz, H-5_{ax}), δ4 0 (1H, *dd*, $J_{5\text{-eq},5\text{-ax}} = 11\,5$ Hz, $J_{5\text{-eq},4} = 4\,9$ Hz, H-5_{eq}), δ2 15, 2 13, 2 07, 2 05 (4 × 3H, 4 × *s*, 4 × MeCOO); β-L-lyxose tetraacetate (≅30%) δ6 02 (1H, *d*, $J_{1,2} = 2.85$ Hz, H-1), δ5 04 (1H, *m*, H-4), δ3 61 (1H, *dd*, $J_{5\text{-ax},5\text{-eq}} = 12\,6$ Hz, $J_{5\text{-ax},4} = 5.1$ Hz, H-5_{ax}), δ4 19 (1H, *dd*, $J_{5\text{-eq},5\text{-ax}} = 12\,6$ Hz, $J_{5\text{-eq},4} = 3.4$ Hz, H-5_{eq}), δ2 12, 2.11, 2 09, 2 08 (4 × 3H, 4 × *s*, 4 × MeCOO), signals of H-2 and H-3 were overlapped with those of α-L-lyxose tetraacetate To obtain the optical rotations of the pure anomers the mixtures (each about 80 mg) were both chromatographically separated (LPLC, H_2O–MeOH, 17·8, flow 2 7 ml/min, fraction size 13 ml, UV monitoring) The β-anomers both D (15 mg) and L (19 mg) eluted first in fractions 76–92, whereas the α-anomers (D 39 mg, L: 47 mg) followed in fractions 111–125 α-L-lyxose tetraacetate· $[\alpha]_D^{20} = -18\,5°$ ($CHCl_3$, *c* 0.95), β-L-lyxose tetraacetate $[\alpha]_D^{20} = +60\,3°$ ($CHCl_3$, *c* 0.64), α-D-lyxose tetraacetate $[\alpha]_D^{20} = +20\,3$ ($CHCl_3$, *c* 0 83), β-D-lyxose tetraacetate· $[\alpha]_D^{20} = -70\,6°$ ($CHCl_3$, *c* 0 82).

HPLC conditions for the comparison of eukovoside and leucosceptoside A Column B, mobile phase H_2O–THF (17 3) containing 0 2% phosphoric acid, flow rate 1 ml/min, detection at 220 nm A mixture (1.1) of the two substances produced a single homogeneous peak (R_t 18 min)

Acknowledgements—This work was supported by grants from the Swiss National Science Foundation and the Deutsche Forschungsgemeinschaft The authors wish to thank Dr Th Schlapfer (Ciba-Geigy AG, Basel) for carrying out the leaf disk bio-assays, J Haas (Department of Food Science, ETH Zurich) for the GC analysis of the aldonitrile acetates of sugars, and Dr T Miyase (Shizuoka College of Pharmacy, Shizuoka, Japan) for providing us with an authentic sample of leucosceptoside A

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