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The Constituents of Conifer Needles. VI.* Phenolic Glycosides from *Pinus sylvestris*

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A β-D-glucopyranoside and an α-L-rhamnopyranoside of 2,3-dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol, an α-L-rhamnopyranoside, a β-D-xylopyranoside and a β-D-glucopyranoside of 1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxypropyl)-2-hydroxyphenoxy]-1,3-propanediol, two β-D-glucopyranosides and one α-L-arabinofuranoside of (+)-isolariciresinol and a β-D-glucoside of (−)-seco-isolariciresinol as well as (+)-catechin and the 3′-*O*-β-D-glucopyranosides of quercetin and 2,3-dihydroquercetin, respectively, have been isolated and identified from needles of *Pinus sylvestris* L.

In previous parts of this series, low-molecular carbohydrates[2,3] and two new diterpene acids[4,5] isolated from the aqueous and methylene chloride soluble part, respectively, of the acetone extract of the needles of *Pinus sylvestris* L, have been studied. From the former fraction, using carbon-Celite chromatography, were also isolated two isomeric β-D-glucopyranosides of guaiacylglycerol,[3] the sugar not being linked to the phenolic group, as in coniferin, but to aliphatic hydroxyls in the glycerol side chain.

In subsequent preliminary studies at the Swedish Forest Products Research Laboratory, using fractionation on Sephadex LH-20 columns (eluent: water and aqueous ethanol) the latter glucosides, together with related glucosides of *p*-hydroxyphenylglycerol, were eluted as a group (which could be further fractionated on an anion-exchange column) after the saccharides.[6] Further studies on these glucosides are now in progress. After the monocyclic glycosides were eluted a group of compounds, which turned out to be dilignol glycosides, followed by a group of known phenolic extractives, of which dihydroquercetin (taxifolin) 3′-*O*-β-D-glucopyranoside seemed rather predominant. The fractionation of these two latter groups and the identification of a series of compounds thus obtained, have now been performed and are reported in this paper. The structural determination of two of the compounds, the 4′-*O*-β-D-glucopyranoside (*1*) and the 4′-*O*-α-L-rhamnopyranoside (*2*) of 2,3-dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol has recently been reported separately.[1] Some

Table 1. Fractionation of part (10.3 g) of the 2-butanone soluble products on Sephadex LH-20. Eluent: water.

Fraction No.	Elution volume/l	Weight/g	Main constituents (compound No.)
I	1.84	2.12	[a]
II	2.24	0.21	*4*
III	2.92	0.28	*4, 5*
IV	4.00	0.31	*1, 15*
V	5.28	0.27	*6, 7, 9, 10*
VI	7.60	0.69	*2, 9, 10*
VII	8.00	0.08	*2, 9*
VIII	9.80	0.19	*8, 17, 3*
IX	13.20	3.93	*17, 3*
X	16.90[b]	0.21	*18, 19*
XI	19.70[b]	0.03	*20*
XII	24.00[c]	0.85	*21*

[a] Sugars and *p*-hydroxyphenyl- and guaiacylglycerol glucosides. [b] 50 % EtOH. [c] 96 % EtOH.

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* Part V. See Ref. 1.

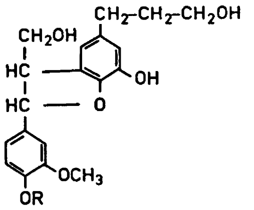
Table 2. Isolated and identified phenolic compounds.

Com-pound	Yields, % of the respective fraction			Total yield %
	Ethyl acetate soluble	2-Butanone soluble	Water soluble	On dry weight of the needles
1	Trace	0.9	2.0	0.17
2	Trace	4.9	3.0	0.39
3	1.1	0.3	Trace	0.05
4	0.6	2.6	2.6	0.29
5	0.6	1.6	1.2	0.16
6	0.9	1.0	0.3	0.09
7	2.0	0.5	Trace	0.09
8	0.3	0.9	0.2	0.06
9	Trace	0.3	0.2	0.03
10	Trace	0.1	0.1	0.01
15		0.3	0.2	0.03
17	6.8	38.8	3.8	2.05
18	2.4	Trace		0.08
19	1.1	Trace		0.04
20	1.7	Trace		0.06
21	1.0			0.03
Total	18.5	51.9	13.4	3.57

of our results on isolation and identification of the phenolic glycosides were recently briefly presented.[7]

RESULTS AND DISCUSSION

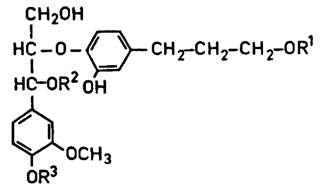
The needles collected in spring were extracted with acetone and divided in fractions of compounds soluble in light petroleum, ethyl acetate, 2-butanone (saturated with water) and water. The phenolic compounds from the three fractions were separated on Sephadex LH-20 columns (eluent: water and aqueous ethanol). Table 1 shows the order in which the compounds from the 2-butanone fraction were eluted. Refractionations were made on silicic acid columns. The compounds isolated and identified are presented in the scheme below, and their approximate yield from the three fractions in Table 2. The identification of compounds *1*, *2* and *3* were reported previously.[1] 2,3-Dihydroquercetin (*19*), quercetin (*20*) and their 3′-*O*-



1 R = β-D-glucopyranoside

2 R = α-L-rhamnopyranoside

3 R = H

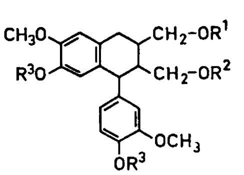


4 $R^1=R^3=H$ $R^2=$ β-D-glucopyranoside

5 $R^1=R^2=H$ $R^3=$ β-D-xylopyranoside

6 $R^2=R^3=H$ $R^1=$ α-L-rhamnopyranoside

7 $R^1=R^2=R^3=H$



8 $R^1=R^3=H$ $R^2=$ α-L-arabinofuranoside

9 $R^2=R^3=H$ $R^1=$ β-D-glucopyranoside

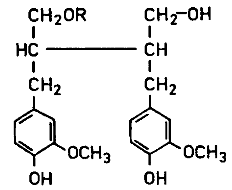
10 $R^1=R^3=H$ $R^2=$ β-D-glucopyranoside

11 $R^1=R^3=CH_3$ $R^2=H$

12 $R^2=R^3=CH_3$ $R^1=H$

13 $R^1=R^2=R^3=CH_3$

14 $R^1=R^2=R^3=H$



15 R = β-D-glucopyranoside

16 R = H

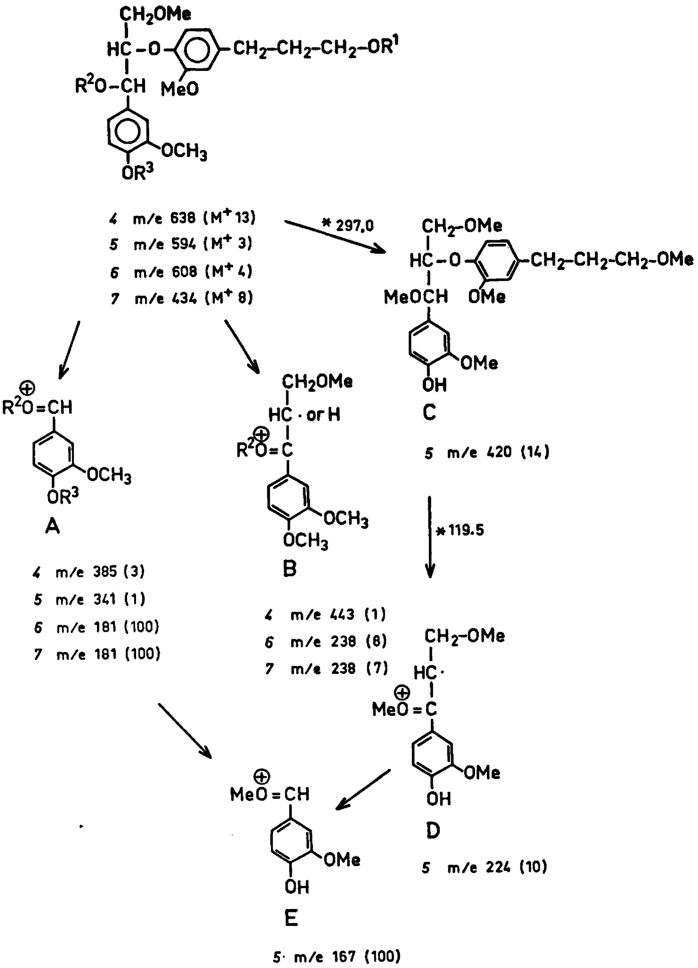


Fig. 1. Mass spectrometric fragments from methylated compounds *4*–*7*.

glucopyranosides *17* and *18* respectively, and (+)-catechin (*21*) were compared with authentic samples and found to be identical in all respects.

The sugar part of the glycosides (which all contain only one sugar unit as shown by NMR, of the free glycosides and their acetates, and by MS of their methyl ethers) were identified as β-D-glucopyranose (in *4*, *9*, *10* and *15*), β-D-xylopyranose (in *5*), α-L-rhamnopyranose (in *6*) and as α-L-arabinofuranose (in *8*); see Experimental.

Compounds *4*–*7* gave the same red colour with diazotized sulfanilic acid, indicating α-hydroxyvanillyl structures. Their ^1H NMR spectra were very similar, showing the presence of six aromatic protons, one benzylic proton (doublet around δ 4.9), one methoxyl group and six protons characteristic of a phenylpropanol side chain (broad triplet around δ 2.6 and multiplets around δ 1.8 and 3.5). Acidic hydrolysis of *4*, *5* and *6* made the aglycone decompose, but the sugars were identified as D-glucose, D-xylose and L-rhamnose, respectively. When hydrolyzed with a commercial enzyme preparation compound *4* and *5* gave the aglycone *7* while compound *6* was unaffected. The latter, however, was found to be identical with the *threo*-isomer of 1-(4-hydroxy-3-methoxyphenyl)-2-[2-hydroxy-4-(3-*O*-α-L-rhamnopyranoside propanol)-phenoxy]-1,3-propanediol (TLC, NMR and MS of the acetate) previously isolated from *Thuja plicata*.[8]

The ^{1}H NMR spectrum of acetylated *4* showed, contrary to those of acetylated *5* and *6*, no downfield shift for the proton, indicating that the glucose in compound *4* is linked to the benzylic alcohol. The ^{1}H NMR spectrum of acetylated *5*, on the other hand, showed only one aromatic acetyl, indicating xylose, that is linked to a phenolic hydroxyl. The structures were further verified by mass spectra of the methylated glycosides and the aglycone (Fig. 1). All derivatives gave peaks corresponding to the expected molecular ions. Fragment A was most significant. The occurrence of *m/e* 385 and *m/e* 341 and absence of *m/e* 181 for compound *4* and *5*, respectively, proved the presence of a hexose-tetra-*O*-methyl and a pentose-tri-*O*-methyl residue in fragment A from *4* and *5*, respectively. The low intensity of *m/e* 341 (1 %) for compound *5*, having the xylose in a phenolic position, is expected, since such a glycosidic linkage is weaker than a linkage to an aliphatic hydroxyl group. Because of this, only MS of compound *5* showed the occurrence of fragment C, D and E, while compounds *4*, *6* and *7* gave fragment B. High resolution mass spectrometry of compounds *4*–*7* established their molecular formulas.

As revealed by the ^{1}H NMR spectrum, the aglycone *7* obtained from compound *4* by enzymatic hydrolysis has a *threo*-configuration in the glycerol chain, as was previously found in the guaiacylglycerol glycosides,[3] while the aglycone from compound *5* contains a mixture of the *threo*- and *erythro*-isomer in the ratio 1:2. Compound *6* has a *threo*-configuration. This was demonstrated in the acetylated compounds by the shifts and coupling constants of the benzylic α-protons, in accordance with Ludwig *et al.*[9] The glycosides *4*–*6* represent a notable example of three different sugars linked to hydroxyl groups in the same aglycone, but in three different positions. We looked carefully for the other combinations without finding any indication of their presence. The saturated state of the side chain and the fact that one of the aromatic rings is catecholic is a notable fact both for this group of compounds and for compounds *1*–*3*.

After hydrolysis, compounds *8*, *9* and *10* gave the same aglycone, (+)-isolariciresinol (*14*) (identical with authentic sample), and L-arabinose (from *8*) and D-glucose (from *9* and *10*). Methylation/hydrolysis studies showed sugar linkage to one of the two aliphatic hydroxyl groups. To verify the position of the arabinose linkage in compound *8*, the glycoside was methylated with methyl iodide in dimethylformamide and sodium hydride,[10] formolysed and hydrolysed. Chromatographic purification of the product yielded compound *11*. This trimethyl ether was not identical with the isomeric compound *12*, synthesized from conidendrin,[6] but they both gave the same product *13* when methylated. This proves that arabinose is linked to the α^3 hydroxyl in compound *8*.

Compound *9* after methylation and hydrolysis yielded a compound identical with the synthesized compound *12*. This proves that the glucose is linked to the α^2 hydroxyl. The structure of compound *10*, which was only obtained in admixture with *9*, was verified by the following findings: in NMR spectra of the mixture two anomeric glucose protons (ratio 2:3) could be seen; at δ 4.26 and 4.30 (CD_3OD), 4.76 and 4.95 (C_5D_5N), respectively, of which the latter must arise from *9*. Mass spectra of the methylated mixture showed an almost identical pattern with that of pure *9* including the molecular ion (*m/e* 620), and by formolysis and hydrolysis of that mixture compound *11* and *12* were obtained in the ratio 2:3, as revealed by the ^{1}H NMR spectra.

After hydrolysis compound *15* gave (−)-seco-isolariciresinol (*16*) (identical with an authentic sample) and D-glucose, and methylation with MeI/DMF, NaH yielded a heptamethyl ether. Since methylation with diazomethane followed by hydrolysis gave the dimethyl ether of *16* (identical with an authentic sample obtained by methylation of *16* with diazomethane) the glucose must be linked to one of the equivalent aliphatic hydroxyl groups.

The chromatographic behaviour of the compounds is shown in Table 3. The characteristic colour reactions on TLC with the three sprays used are, with one exception (compound *17*) almost identical for a glycoside and its aglycone. The anomeric configuration and ring size of the sugars in the glycosides *4*, *5*, *8*, *9*, *10*, and *15* were verified by ^{1}H NMR. The coupling constant of the anomeric proton in the D-glucopyranosides *4*, *9*, *10* and *15* and in

Table 3. Chromatographic mobilities (TLC in 2-butanone saturated with water) and colour reactions.

Compound	R_V[a]	Colour		
		Spray *a*	Spray *b*	Spray *c*
1	0.25	Dark red		Red violet
2	0.47	Dark red		Red violet
3	0.88	Dark red		Red violet
4	0.33	Red		Blue violet
5	0.40	Red		Blue violet
6	0.61	Red		Violet
7	0.88	Red		Blue violet
8	0.59	Brown		Red
9	0.36	Brown		Red
10	0.36	Brown		Red
14	0.86	Brown		Red
15	0.40	Green		Red violet
16	0.90	Green		Red violet
17	0.53	Yellow	Red violet	Orange yellow
18	0.51	Yellow brown	Green gray	Yellow
19	0.99	Yellow	Blue violet	Orange yellow
20	1.01	Pale yellow	Green gray	Yellow
21	0.97	Yellow	Green	Red orange

[a] Mobilities relative to that of vanillin.

the D-xylopyranoside *5* were all around 7 Hz thus indicating *β*-D-pyranosides. The coupling constant of the anomeric proton in the L-arabinofuranose *8*, was 2.0 Hz, indicating an α-L-arabinofuranoside.[11] Besides, the chemical shifts for the anomeric protons in compounds *8* and *9*, *10* and *15*, respectively, were very similar to those previously reported for methyl-α-L-arabinofuranoside and methyl-*β*-D-glucopyranoside,[11] respectively. The chemical shift and coupling constant for the anomeric proton in compound *5* were similar to those of authentic phenyl-*β*-D-xylopyranoside in C_6D_5N (δ 5.41, J 7.0 Hz).

To our knowledge compounds *1*–*5*, *7*, *8*, *11*–*13* and *15* have not previously been reported by other authors. Compounds *9* and/or *10* (the exact position of glucose not established) has previously been reported.[16]

EXPERIMENTAL

General

TLC was generally performed on Silica Gel HF_{254} (Merck) with 2-butanone, saturated with water, as solvent (mobilities in Table 3) and for further establishing of the purities in chloroform-methanol-water (70:30:5), ethyl acetate-methanol-water (130:17:13) and chloroform-isopropanol-water (60:25:2). Column chromatography was run on Sephadex LH-20, with water and water-ethanol as solvent and on silicic acid (Mallinckrodt, 100 mesh) with (*a*) water-saturated 2-butanone, (*b*) chloroform-methanol-water (70:25:3) and (*c*) ethyl acetate-acetone (1:1) as solvent. TLC-plates (after inspection in UV light) were sprayed with (*a*) 0.1 % diazotized sulfanilic acid in 10 % sodium carbonate followed by 50 % sulfuric acid, (*b*) 1 % iron(III) chloride in water and (*c*) anisaldehyde-sulfuric acid-ethanol (1:1:18), followed by heating (140 °C). Low and high resolution mass spectra were recorded at 70 *e*V or 30 *e*V on Varian MAT CH 7 and AEI 902 instruments, respectively, the latter spectra at the Institute of Medical Biochemistry, University of Gothenburg. The ^{1}H NMR spectra were recorded at 100 MHz on a Varian HA-100 D instrument, equipped with a Varian VFT-100 Fourier Transform System.

Isolation

Fresh needles (1.2 kg; water content 37 %) from *Pinus sylvestris* L, collected in spring, were boiled with acetone for 30 min. After filtration, the needles were milled and extracted continuously with acetone in a Soxhlet apparatus (2 × 24 h). After evaporation of the acetone, the combined acetone extracts were diluted with water (1.5 l) and extracted with light petroleum, b.p. 40–60 °C, (3 × 0.5 l), ethyl acetate (3 × 0.5 l) and 2-butanone (3 × 0.5 l). The yields of the fractions after evaporation were: 60.4 g (light petroleum), 23.6 g (ethyl acetate), 31.1 g (2-butanone) and 48.7 g (water).

Part of the 2-butanone fraction (10.3 g) was added to a Sephadex LH-20 column, and eluted with water and ethanol/water; 12 fractions were collected (weights and contents are given in Table 1). The fractions were then fractionated on silicic acid columns with solvent *a*, except fractions III (solvent *b*), V and VII (solvent *c*)

In order to obtain pure samples of *6* and *8*, crude subfractions of the respective compound were treated with an enzyme (see below) which hydrolysed most interfering compounds but left *6* and *8* unchanged, followed by column separations (solvent *a*) yielding pure *8* and *6*, respectively.

The same procedure was used for the rest of the 2-butanone fraction, for the ethyl acetate fraction and for the water fraction. Table 2 gives the content of the compounds in the different fractions and their total approximate yield calculated on the dry weight of the needles (based on the weights of the fractions and visual TLC estimations). All the compounds isolated were chromatographically pure (TLC) in several solvent systems but attempts to crystallize any of the glycosides failed.

Hydrolysis

Compounds *4*, *5*, *9*, *10*, *15*, *17* and *18* were hydrolysed enzymatically with a commercial crude enzyme (cellulase C 36, Rohm and Haas Co.). Enzyme (about 5 mg) was added to the substance (50 mg) in water (50 ml), and the mixture was left at room temperature overnight. By extraction with ethyl acetate (4 × 25 ml), drying (Na_2SO_4) and evaporation, an almost pure aglycone was obtained from each glycoside. In the aqueous fraction the sugar was identified by optical rotation (the weights corresponded to one sugar unit per glucoside) and by GLC as its trimethylsilyl ether and as its alditol acetate;[12,13] D-glucose from compounds *4*, *9*, *10* and *15* and D-xylose from compound *5*. Compounds *6* and *8*, which were not affected by the enzyme, were hydrolysed with 1 M sulfuric acid for 3 h at 100 °C. Extraction as above gave (+)-isolariciresinol (identical with an authentic sample; IR, MS, NMR) and L-arabinose from compound *8*, but a complex of phenolic products and L-rhamnose from compound *6*.

Methylation

About 10 mg of each glycoside was methylated with methyl iodide in dimethylformamide and sodium hydride.[10] The *O*-methyl derivatives were purified on TLC (ethyl acetate as solvent). Pure derivatives were obtained from all glycosides except from compounds *17* and *18*, where decomposition occurred under the conditions used. The products obtained from the methylated glycosides after formolysis, hydrolysis, subsequent reduction and acetylation were studied by GLC/MS.[14,15] Thereby 2,3,-4,6-tetra-*O*-methyl-1,5-di-*O*-acetyl-glucitol was identified from compounds *4*, *9* and *10*, 2,3,-5-tri-*O*-methyl-1,4-di-*O*-acetyl-arabinitol from compound *8*, 2,3,4-tri-*O*-methyl-1,5-di-*O*-acetyl-rhamnitol from compound *6* and 2,3,4-tri-*O*-methyl-1,5-di-*O*-acetyl-xylitol from compound *5*, thus verifying the furanoside form of arabinose and the pyranoside form of the other sugars.

Compound 4 (amorphous), $[\alpha]_D^{20}$ −7.1° (*c* 3.0, EtOH). ^1H NMR (CD_3OD): δ 1.65 − 1.95 (2 H, m), 2.55 (2 H, broad t, *J* 7 Hz), 3.1 − 3.9 (10 H, m), 3.81 (3 H, s), 4.1 − 4.4 (1 H, m), 4.55 (1 H, d, *J* 7.0 Hz), 6.5 − 7.2 (6 H, m).

Acetylation (Ac_2O/pyr) of compound *4* yielded the octa-acetate (amorphous). ^1H NMR ($CDCl_3$): δ 1.80 (3 H, s), 1.91 (3 H, s), 1.95 (9 H, broad s), 2.02 (3 H, s), 2.24 (3 H, s), 2.26 (3 H, s), 1.8 − 2.4 (2 H, m), 2.62 (2 H, broad t, *J* 7 Hz), 3.4 − 3.7 (1 H, m), 3.81 (3 H, s), 3.6 − 4.3 (6 H, m), 4.52 (1 H, m), 4.7 − 5.2 (5 H, m), 6.7 − 7.1 (6 H, m).

Methylation (CH_3I/DMF, NaH) of compound *4* yielded the octamethyl ether (amorphous). MS, [IP 30 eV; *m/e* (% rel. int.)]: 638 (M, 14), 594 (1), 502 (1), 472 (6), 443 (1), 403 (1), 385 (3), 371 (2), 313 (2), 252 (1), 245 (3), 233 (1), 222 (25), 219 (13), 218 (12), 208 (11), 188 (11), 187 (100), 177 (10), 163 (17), 155 (12), 151 (14), 127 (9), 115 (9), 111 (30), 101 (35), 89 (16), 75 (17), 71 (16), 45 (45). Mol. wt., obs. 638.330, calc. for $C_{33}H_{50}O_{12}$ 638.330.

Compound 5 (amorphous). Mixture of *threo* and *erythro*-isomer in ratio 1:2. ^1H NMR (CD_3OD): δ 1.7 − 1.9 (2 H, m), 2.52 (2 H, broad t, *J* 7 Hz), 3.3 − 4.0 (9 H, m), 3.79 (3 H, s), 4.15 (1 H, m), 4.91 (2/3 H, d, *J* 5.0 Hz), 4.97 (1/3 H, d, *J* 6.0 Hz), 6.49 (2/3 H, dd, *J* 2.2 and 8.4 Hz), 6.54 (1/3 H, dd, *J* 2.2 and 8.4 Hz), 6.6 − 7.1 (5 H, m).

Acetylation (Ac_2O/pyr) of compound *5* yielded the heptaacetate (amorphous). ^1H NMR ($CDCl_3$): δ 1.95 (3 × 1/3 H, s), 1.97 (3 × 2/3 H, s), 2.02 (3 H, s), 2.04 (9 H, broad s), 2.09 (3 H, s), 2.15 (3 × 2/3 H, s), 2.25 (3 × 1/3 H, s), 1.8 − 2.3 (2 H, m), 2.62 (2 H, broad t, *J* 7 Hz), 3.47 (1 H, dd, *J* 7.6 and 12.0 Hz), 3.7 − 3.9 (1 H, m), 3.9 − 4.5 (4 H, m), 4.23 (1 H, dd, *J* 4.4 and 12.0 Hz), 4.67 (1 H, m), 4.9 − 5.3 (4 H, m), 5.97 (2/3 H, d, *J* 4.4 Hz), 6.02 (1/3 H, d, *J* 6.5 Hz) 6.75 − 7.15 (6 H, m).

As comparison, phenyl-β-D-tri-*O*-acetyl-xylopyranoside, available in this laboratory: ^1H NMR ($CDCl_3$): δ 2.07 (9 H, broad s), 3.52 (1 H, dd, *J* 7.6 and 12.0 Hz), 4.23 (1 H, dd, *J* 4.6 and 12.0 Hz), 4.9 − 5.3 (4 H, m), 6.9 − 7.4 (5 H, m).

Methylation (CH_3I/DMF, NaH) of compound *5* yielded the heptamethyl ether (amorphous) MS, [IP 30 eV; *m/e* (% rel. int.)]: 594 (M, 3), 420 (14), 239 (1), 224 (10), 222 (11), 193 (7), 179 (5), 175 (6), 174 (6), 168 (10), 167 (100), 151 (8), 137 (8), 125 (8), 101 (29), 99 (15), 45 (28). Mol. wt., obs 594.304, calc for $C_{31}H_{46}O_{11}$ 594.304.

Compound 6 (amorphous), $[\alpha]_D^{20}$ −58.1° (*c* 2.8, EtOH). ^1H NMR (CD_3OD): δ 1.24 (3 H, d, *J* 6.0 Hz), 1.7−2.0 (2 H, m), 2.59 (2 H, broad t, *J* 7 Hz), 3.4−3.9 (8 H, m), 3.79 (3 H, s), 4.0−4.2 (1 H, m), 4.63 (1 H, d, *J* 1.5 Hz), 4.91 (1 H, d, *J* 6.5 Hz), 6.55 (1 H, dd, *J* 2.0 and 8.4 Hz), 6.68 (1 H, d, *J* 2.0 Hz), 6.74 (1 H, d, *J* 8.4 Hz), 6.86 (1 H, dd, *J* 1.8 and 8.0 Hz), 6.94 (1 H, d, *J* 8.0 Hz), 7.01 (1 H, d, *J* 1.8 Hz).

Acetylation (Ac_2O/pyr) of compound *6* yielded the hepta-acetate (amorphous). ^1H NMR ($CDCl_3$): δ 1.21 (3 H, d, *J* 6.0 Hz), 1.95 (3 H, s), 1.99 (3 H, s), 2.05 (3 H, s), 2.06 (3 H, s), 2.15 (3 H, s), 2.26 (3 H, s), 2.30 (3 H, s), 1.8−2.3 (2 H, m), 2.66 (2 H, broad t, *J* 7 Hz), 3.4−4.3 (5 H, m), 4.6−4.8 (1 H, m), 4.71 (1 H, broad s), 5.06 (1 H, t, *J* 10.0 Hz), 5.24 (1 H, dd, *J* 1.8 and 3.5 Hz), 5.33 (1 H, dd, *J* 3.5 and 10.0 Hz), 6.06 (1 H, d, *J* 6.5 Hz), 6.8−7.0 (6 H, m).

Methylation (CH_3I/DMF, NaH) of compound *6* yielded the heptamethyl ether (amorphous). MS [IP 30 eV; *m/e* (% rel. int.)]: 608 (M, 4), 383 (7), 238 (7), 207 (7), 182 (11), 181 (100), 151 (20), 131 (12), 119 (12), 101 (21), 88 (17), 69 (62), 59 (11), 45 (40). Mol. wt., obs. 608.321, calc. for $C_{32}H_{48}O_{11}$ 608.320.

Compound 7. The *threo*-isomer obtained from compound *4* (amorphous). $[\alpha]_D^{20}$ +35.4°, (*c* 0.7, EtOH). ^1H NMR (CD_3OD): δ 1.4−1.9 (2 H, m), 2.55 (2 H, broad t, *J* 7 Hz), 3.47 (1 H, dd, *J* 5.0 and 11.5 Hz), 3.54 (2 H, t, *J* 6.5 Hz), 3.75 (1 H, dd, *J* 4.0 and 11.5 Hz), 3.81 (3 H, s), 3.9−4.2 (1 H, m), 4.91 (1 H, d, *J* 6.5 Hz), 6.55 (1 H, dd, *J* 2.2 and 8.2 Hz), 6.67 (1 H, d, *J* 2.2 Hz), 6.91 (1 H, d, *J* 8.2 Hz), 6.74 (1 H, d, *J* 8.0 Hz), 6.86 (1 H, dd, *J* 1.8 and 8.0 Hz), 7.01 (1 H, d, *J* 1.8 Hz). In the ^1H NMR spectra of the *erythro*-, *threo*-mixture of *7* obtained from compound *5* or isolated from fraction V (Table 1), slight differences could be seen between the two isomers when compared with the pure *threo*-isomer above, arising from the *erythro*-isomer; δ (CD_3OD): 3.9−4.3 (1 H, m), 4.86 (1 H, d, *J* 5.0 Hz), 6.50 (1 H, dd, *J* 2.2 and 8.2 Hz), 6.86 (1 H, d, *J* 8.0 Hz).

Acetylation (Ac_2O/pyr) of compound *7* *threo*-isomer yielded the pentaacetate (amorphous). ^1H NMR ($CDCl_3$): δ 1.92 (3 H, s), 2.02 (3 H, s), 2.04 (3 H, s), 2.23 (3 H, s), 2.27 (3 H, s), 1.9−2.4 (2 H, m), 2.63 (2 H, broad t, *J* 7 Hz), 3.80 (3 H, s), 4.0−4.2 (4 H, m), 4.5−4.8 (1 H, m), 6.05 (1 H, d, *J* 7.0 Hz), 6.8−7.0 (6 H, m). In the ^1H NMR spectra of acetylated (Ac_2O/pyr) isomer mixture mentioned above the *erythro*-isomer could be seen; δ ($CDCl_3$): 1.96 (3 H, s), 2.02 (3 H, s), 2.10 (3 H, s), 2.12 (3 H, s), 2.27 (3 H, s), 1.9−2.4 (2 H, m), 2.61 (2 H, broad t, *J* 7 Hz), 3.20 (3 H, s), 4.08 (1 H, dd, *J*, 4.0 and 12.0 Hz), 4.36 (1 H, dd, *J* 5.5 and 12.0 Hz), 4.5−4.8 (1 H, m), 6.01 (1 H, d, *J* 5.0 Hz), 6.8−7.0 (6 H, m).

Methylation (CH_3I/DMF, NaH) of compound *7* yielded the pentamethyl ether (amorphous) MS [IP 70 eV; *m/e* (% rel. int.)]: 434 (M, 8), 238 (7), 207 (7), 182 (12), 181 (100), 151 (12), 45 (12). Mol. wt., obs 434.230, calc. for $C_{24}H_{34}O_7$ 434.230.

Compound 8 (amorphous), $[\alpha]_D^{20}$ −36.8° (*c* 1.0, EtOH). ^1H NMR (CD_3OD): δ 1.75−2.25 (2 H, m), 2.81 (2 H, broad d, *J* 7 Hz), 3.13 (1 H, broad dd, *J* 2 and 10 Hz), 3.5−4.1 (9 H, m), 3.76 (3 H, s), 3.78 (3 H, s), 4.73 (1 H, d, *J* 1.8 Hz), 6.18 (1 H, s), 6.59 (1 H, dd, *J* 2.0 and 8.0 Hz), 6.64 (1 H, s), 6.67 (1 H, d, *J* 2.0 Hz), 6.75 (1 H, d, *J* 8.0 Hz).

Acetylation (Ac_2O/pyr) of compound *8* yielded the hexa-acetate (amorphous). ^1H NMR ($CDCl_3$): δ 2.05 (6 H, s), 2.07 (3 H, s), 2.08 (3 H, s), 2.20 (3 H, s), 2.28 (3 H, s), 1.8−2.4 (2 H, m), 2.90 (2 H, broad d, *J* 7 Hz), 3.14 (1 H, broad dd, *J* 2 and 10 Hz), 3.78 (1 H, broad dd, *J* 3 and 10 Hz), 3.76 (3 H, s), 3.79 (3 H, s), 4.0−4.5 (6 H, m), 4.88 (1 H, broad s), 4.90−5.05 (1 H, m), 5.07 (1 H, d, *J* 1.8 Hz), 6.42 (1 H, s), 6.6−6.8 (3 H, m), 6.96 (1 H, d, *J* 8.2 Hz).

Methylation (CH_3I/DMF, NaH) of compound *8* yielded the hexamethyl ether (amorphous). MS [IP 70 eV; *m/e* (% rel. int.)]: 576 (M, 8), 416 (2), 401 (27), 384 (10), 383 (100), 369 (17), 353 (12), 351 (18), 339 (12), 307 (7), 269 (6), 201 (5), 165 (6), 151 (24), 115 (5), 111 (5), 101 (27), 71 (13), 57 (13), 55 (10), 45 (26).

Compound 9 (amorphous) $[\alpha]_D^{20}$ +8.0° (*c* 0.9, EtOH). ^1H NMR (CD_3OD): δ 1.6−2.0 (1 H, m), 2.0−2.4 (1 H, m), 2.84 (2 H, broad d, *J* 7 Hz), 3.2−4.1 (11 H, m), 3.78 (3 H, s), 3.80 (3 H, s), 4.30 (1 H, d, *J* 7.5 Hz), 6.20 (1 H, s), 6.5−6.7 (2 H, m), 6.65 (1 H, s), 6.75 (1 H, d, *J* 8.0 Hz). ^1H NMR of the acetylated (Ac_2O/pyr) compound *9* as well as that of the acetylated compounds *9* and *10* mixture were in agreement with that of (+)-isolariciresinol-D-glucoside previously [16] reported.

Methylation (CH_3I/DMF, NaH) of compound *9* yielded the heptamethyl ether (amorphous). MS [IP 70 eV; *m/e* (% rel. int.)]: 620 (M, 20), 402 (14), 401 (49), 385 (12), 384 (49), 383 (100), 370 (11), 369 (39), 353 (23), 351 (16), 339 (22), 201 (10), 165 (11), 151 (46), 149 (34), 127 (12), 121 (12), 115 (10), 111 (16), 101 (44), 98 (14), 97 (14), 89 (18), 88 (65), 85 (18), 83 (19), 75 (48), 73 (24), 72 (45), 71 (54), 70 (18), 69 (34), 59 (14), 57 (78), 55 (37), 45 (75).

Compound 10 only obtained in admixture with compound *9*, showed a very similar ^1H NMR to that of pure *9*. Two anomeric sugar protons, however, could be seen at δ 4.28 and 4.30 (CD_3OD) or at δ 4.76 and 4.95 (C_5D_5N).

Compound 11. Crystals after storage, m.p. 141 – 155 °C; $[\alpha]_D^{20}$ – 10.0° (*c* 1.0, $CHCl_3$); MS, [IP 70 eV; *m/e* (% rel. int.)]: 403 (25), 402 (M, 100), 370 (7), 353 (19), 340 (14), 339 (56), 325 (9), 324 (8), 321 (12), 312 (16), 299 (10), 269 (34), 201 (13), 189 (19), 151 (24), 45 (25). ^1H NMR ($CDCl_3$): δ 1.5 – 1.9 (1 H, m), 2.0 – 2.4 (1 H, m), 2.74 (2 H, broad d, *J* 7.5 Hz), 3.3 – 3.9 (4 H, m), 3.39 (3 H, s), 3.57 (3 H, s), 3.79 (3 H, s), 3.84 (3 H, s), 3.87 (3 H, s), 3.98 (1 H, broad d, *J* 10.4 Hz), 6.23 (1 H, s), 6.58 (1 H, s), 6.61 (1 H, d, *J* 1.7 Hz), 6.75 (1 H, dd, *J* 1.7 and 8.2 Hz), 6.81 (1 H, d, *J* 8.2 Hz).

Compound 12. Obtained from conidendrin[6] after methylation (CH_3I/DMF, NaH) and reduction ($LiAl_4$). Crystallized from ethanol, m.p. 140.5 – 141.0 °C, $[\alpha]_D^{20}$ +25.5° (*c* 2.8, $CHCl_3$). Anal. $C_{23}H_{30}O_6$: C, H. MS [IP 70 eV; *m/e* (% rel. int.)]: 403 (22), 402 (M, 84), 370 (12), 340 (30), 339 (100), 324 (10), 312 (20), 269 (24), 201 (16), 189 (18), 176 (13), 165 (13), 152 (10), 151 (42), 45 (78), 43 (19). ^1H NMR ($CDCl_3$): δ 1.8 – 2.1 (2 H, m), 2.6 – 3.1 (2 H, m), 3.25 (1 H, dd, *J* 5.2 and 10.0 Hz), 3.25 (3 H, s), 3.44 (1 H, dd, *J* 2.0 and 10.0 Hz), 3.56 (3 H, s), 3.80 (3 H, s), 3.83 (3 H, s), 3.88 (3 H, s), 3.6 – 3.9 (3 H, m), 6.19 (1 H, s), 6.60 (2 H, broad s), 6.70 (1 H, dd, *J* 2.0 and 8.0 Hz), 6.83 (1 H, d, *J* 8.0 Hz).

Compound 13. Obtained from compounds *11*, *12* and *14* by methylation (CH_3I/DMF, NaH). Crystallized from methanol, m.p. 110.4 – 111.9 °C; $[\alpha]_D^{20}$ – 8.5° (*c* 0.9, $CHCl_3$). Anal. $C_{24}H_{32}O_6$: C, H. MS [IP 70 eV; *m/e* (% rel. int.)] 417 (28), 416 (M, 100), 384 (11), 354 (14), 353 (49), 340 (22), 339 (45), 324 (12), 321 (21), 312 (27), 308 (10), 299 (10), 269 (28), 208 (18), 201 (22), 181 (10), 176 (15), 165 (11), 152 (10), 151 (39), 45 (63). ^1H NMR ($CDCl_3$): δ 1.7 – 2.0 (1 H, m), 2.00 – 2.35 (1 H, m), 2.85 (2 H, broad d, *J* 7.8 Hz), 3.09 (1 H, dd, *J* 3.3 and 9.5 Hz), 3.26 (3 H, s), 3.35 (3 H, s), 3.39 (1 H, dd, *J* 3.3 and 9.5 Hz), 3.46 (2 H, d, *J* 5.0 Hz), 3.58 (3 H, s), 3.80 (3 H, s), 3.83 (3 H, s), 3.87 (3 H, s), 4.00 (1 H, broad d, *J* 10.5 Hz), 6.26 (1 H, s), 6.62 (1 H, s), 6.63 (1 H, d, *J* 1.8 Hz), 6.70 (1 H, dd, *J* 1.8 and 7.8 Hz), 6.82 (1 H, d, *J* 7.8 Hz).

Compound 14. Identical (m.p., IR, MS, NMR) with an authentic sample of (+)-isolariciresinol, m.p. 115 – 116 °C (lit. value 112 °C;[17] 115 – 117 °C[16]): $[\alpha]_D^{20}$ +63.3° (*c* 2.0, $(CH_3)_2C=O$) (lit. value +61.7 °C;[16] +69.4 °C[17]) MS [IP 70 eV; *m/e* (% rel. int.)]: 361 (32), 360 (M, 100), 342 (12), 341 (10), 325 (11), 312 (18), 311 (97), 297 (11), 284 (27), 279 (12), 271 (16), 255 (12), 241 (29), 211 (10), 197 (10), 187 (14), 175 (27), 137 (33), 131 (10), 115 (15), 55 (12). ^1H NMR (CD_3OD): δ 1.6 – 2.2 (2 H, m), 2.77 (2 H, broad d, *J* 7 Hz), 3.4 – 3.9 (5 H, m), 3.75 (3 H, s), 3.78 (3 H, s), 6.20 (1 H, s), 6.51 (1 H, dd, *J* 2.0 and 8.0 Hz), 6.64 (1 H, s), 6.68 (1 H, d, *J* 2.0 Hz), 6.75 (1 H, d, *J* 8.0 Hz).

Compound 15 (amorphous), $[\alpha]_D^{20}$ – 20.5° (*c* 3.2, EtOH). ^1H NMR (CD_3OD): δ 1.8 – 2.3 (2 H, m), 2.45 – 2.80 (4 H, m), 3.1 – 4.0 (10 H, m), 3.74 (6 H, s), 4.20 (1 H, d, *J* 7.0 Hz), 6.45 – 6.80 (6 H, m).

Methylation (MeI/DMF, NaH) of compound *15* yielded the heptamethyl ether (amorphous). MS [IP 30 eV; *m/e* (% rel. int.)]: 622 (M, 6), 386 (8), 355 (10), 247 (16), 233 (18), 203 (10), 187 (13), 177 (8), 167 (9), 165 (5), 152 (21), 151 (100), 127 (6), 111 (14), 101 (29), 89 (10), 88 (29), 75 (13), 71 (14), 45 (41).

Compound 16. Identical (NMR, MS, $[\alpha]_D^{20}$) with an authentic sample of (–)-seco-isolariciresinol (spectral data previously reported[18]).

Compound 17. Identical (IR, NMR) with an authentic sample of dihydroquercetin-3′-*O*-*β*-D-glucopyranoside. Amorphous white powder $[\alpha]_D^{20}$ – 32.0° (*c* 0.6, H_2O) (lit.[19] value – 23°). ^1H NMR (CD_3OD): δ 3.3 – 4.0 (6 H, m), 4.55 (1 H, d, *J* 12.0 Hz), 4.98 (1 H, d, *J* 12.0 Hz), 5.88 (1 H, d, *J* 2.0 Hz), 5.93 (1 H, d, *J* 2.0 Hz), 6.88 (1 H, d, *J* 8.0 Hz), 7.10 (1 H, dd, *J* 2.0 and 8.0 Hz), 7.39 (1 H, d, *J* 2.0 Hz). The anomeric sugar proton was hidden under solvent peak at δ 4.8 – 5.0.

Compound 18. Identical (m.p., IR) with quercetin-3′-*O*-*β*-D-glucopyranoside, synthesized from compound *17*.[19] Crystallized from aqueous acetone, m.p. 237.0 – 239.0 °C (lit.[19] value 216 °C).

Compounds 19, 20 *and* 21 were identical (TLC, m.p., IR) with authentic samples of dihydroquercetin (taxifolin), quercetin and (+)-catechin, respectively, available in this laboratory.

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REFERENCES

1. Popoff, T. and Theander, O. *Phytochemistry 14* (1975) 2065.
2. Assarsson, A. and Theander, O. *Acta Chem. Scand. 12* (1958) 1319.
3. Theander, O. *Acta Chem. Scand. 19* (1965) 1792.
4. Enzell, C. and Theander, O. *Acta Chem. Scand. 16* (1962) 607.
5. Norin, T., Sundin, S. and Theander, O. *Acta Chem. Scand. 25* (1971) 607.
6. Popoff, T. and Theander, O. *Unpublished results.*
7. Popoff, T. and Theander, O. *Applied Polymer Symposium 28* (1976) 1341.
8. Manners, G. D. and Swan, E. P. *Can. J. Chem. 49* (1971) 3607.
9. Ludwig, C. H., Nist, B. J. and McCarthy, J. L. *J. Am. Chem. Soc. 86* (1964) 1186.

10. Brimacombe, J. S., Jones, B. D., Stacey, M. and Williard, J. J. *Carbohydr. Res. 2* (1966) 167.
11. Capon, B. and Thacker, D. *Proc. Chem. Soc. London* (1964) 369.
12. Sweely, C. C., Bentley, R., Makita, M. and Wells, W. W. *J. Am. Chem. Soc. 85* (1963) 2497.
13. Sawardeker, J. S., Sloneker, J. H. and Jeanes, A. R. *Anal Chem. 37* (1965) 1602.
14. Hellerqvist, C. G., Lindberg, B., Svensson, S., Holme, T. and Lindberg, A. A. *Carbohydr. Res. 8* (1968) 43.
15. Björndal, H., Hellerqvist, C. G., Lindberg, B. and Svensson, S. *Angew. Chem. 82* (1970) 643.
16. Thieme, H. and Benecke, R. *Pharmazie 24* (1969) 576.
17. Haworth, R. D. and Wilson, L. *J. Chem. Soc.* (1950) 71.
18. Andersson, R., Popoff, T. and Theander, O. *Acta Chem. Scand. B 29* (1975) 835.
19. Hergert, H. L. and Goldschmid, O. *J. Org. Chem. 23* (1958) 700.

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