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TWO GLYCOSIDES OF A NEW DILIGNOL FROM *PINUS SILVESTRIS**

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This paper is dedicated to Professor Karl Kratzl, Vienna, in honour of his 60th birthday.

Key Word Index—*Pinus silvestris*; Pinaceae; dilignol glycosides; 2,3-dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol 4′-*O*-β-D-glucopyranoside and 4′-*O*-α-L-rhamnopyranoside.

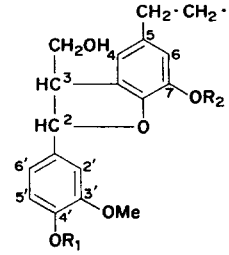
Abstract—The 4′-*O*-β-D-glucopyranoside and the 4′-*O*-α-L-rhamnopyranoside of 2,3-dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol have been isolated and identified. Also isolated were two D-glucosides and an L-arabinoside of (+)-isolariciresinol and a L-rhamnoside, a D-xyloside and a D-glucoside of 1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxypropyl)-2-hydroxyphenoxy]-1,3-propanediol.

INTRODUCTION

Using chromatography on Sephadex LH-20 and ion exchange resin, a series of isomeric *O*-β-D-glucopyranosides of guaiacylglycerol and *p*-hydroxyphenylglycerol (it is notable that in all of them glucose is linked to an aliphatic hydroxyl position), 3′-*O*-glucopyranosides of quercetin and 2,3-dihydroquercetin and a group of dilignol glycosides have been isolated [1] from needles of *Pinus silvestris* L. An account of the fractionation of the dilignol glycosides and the identification of three of them as two *O*-β-D-glucopyranosides and an *O*-α-L-arabino furanoside respectively of (+)-isolariciresinol and three of them as an *O*-L-α-rhamnoside (previously isolated from *Thuja plicata*) [2], an *O*-β-D-xylopyranoside and an *O*-β-D-glucopyranoside respectively of 1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxypropyl)-2-hydroxyphenoxy]-1,3-propanediol will be published elsewhere. The present publication reports the structural determination of two dilignol glycosides (**1** and **2**) also isolated from the mixture and having an aglycone, which to our knowledge is not previously reported.

RESULTS AND DISCUSSION

Both glycoside **1** ($[\alpha]_D^{25}$ −36°) and glycoside **2** ($[\alpha]_D^{25}$ −65°) were obtained amorphous, but chromatographically homogenous. Acid hydrolysis yielded D-glucose and L-rhamnose from **1** and **2** respectively, and from both the aglycone **3** (mp 137–139°; $[\alpha]_D^{25}$ −5·4°) corresponding to the formula $C_{19}H_{22}O_6$ by elemental analysis, was obtained. MS of **3** (showing a strong peak corresponding to the molecular ion) as well as NMR of the acetate were in agreement with the suggested structure. By methylation of **1** and **2** with diazomethane, followed by acid hydrolysis, dihydrodehydrodiconiferyl alcohol (**4**), a known model compound in lignin biosynthesis, was obtained. This proves the structure of **3** as 2,3-dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol and also that the sugars in **1** and **2** are linked to the phenolic group in the 4′-position. The fact that only one sugar unit was linked in each glycoside was evident by determination of the amount of sugar in the hydrolysates. Further, by MS of fully methy-



(**1**) R_1 = Glc; R_2 = H
(**2**) R_1 = Rha; R_2 = H
(**3**) R_1 = R_2 = H
(**4**) R_1 = H; R_2 = Me

* Part 5 in the series "The Constituents of Conifer Needles". Part 4 in (1971) *Acta Chem. Scand.* **25**, 607.

lated glycosides, peaks corresponding to the expected molecular ions, M^+ 606 from **1** and M^+ 576 from **2** were obtained. By GC–MS of the products obtained by formolysis and hydrolysis of the methylated glycosides, followed by reduction and acetylation—the modern technique for structural polysaccharide studies [3,4]—the pyranoside form of the sugars was verified. That **1** contains a β-D-glucosidic and **2** and a α-L-rhamnosidic linkage is supported by their optical rotations. Moreover, compound **1** was hydrolyzed by β-glucosidase. The saturated state of the side chains of the lignol units is notable and also the fact that one of the aromatic rings is catecholic.

EXPERIMENTAL

Needles of *Pinus silvestris* L., collected in spring (only those of previous years), were extracted without drying for 1 hr with boiling Me_2CO. After filtration the needles were dried, milled and extracted 2× with Me_2CO in a Soxhlet for 2 days. The Me_2CO extracts were combined, evaporated to a small vol., some water added, and the suspension obtained extracted several times with (1), petrol (bp 40–60°), (2) EtOAc and (3) 2-butanone, saturated with H_2O. Part of fraction 3 was fractionated on a Sephadex LH-20 column (elution with H_2O and aq. EtOH of increasing EtOH content). Twelve main fractions were collected; from fractions 4 and 6, by subfraction on a silicic acid column (elution with 2-butanone, saturated with H_2O), compounds 1 and 2 were obtained in a chromatographically pure form. The yields corresponded to 0·2 and 0·4% respectively of the dry weight. TLC studies were made on Si gel HF_{254} plates, which were sprayed with 0·1% diazotized sulphanilic acid in 10% Na_2CO_3 followed by 50% H_2SO_4. The TLC system was *n*-butanone, saturated with H_2O. The R_v values given are migrations relative to vanillin. NMR data were taken at 100 MHz and tetramethylsilane was used as an internal standard, and *s*, *d*, *m*, *q* and *t* denote singlet, doublet, multiplet, quartet and triplet respectively.

Compound 1. Amorphous, $[\alpha]_D^{25}$ −35·6° (*c* 1, EtOH). R_v 0·25 (colour: dark red). NMR (CD_3OD) δ 1·6–2·0 *m* (2H); 2·62 *t*, *J* 7·0 Hz (2H); 3·2–4·0 *m* (11H); 3·76 *s* (3H); 5·50 *d*, *J* 5·6 Hz (1H); 6·54 broad *s* (2H); 6·90 *q*, *J* 2·0 Hz, *J* 8·5 Hz (1H); 7·01 *d*, *J* 2·0 Hz (1H); 7·10 *d*, *J* 8·5 Hz (1H). The anomeric sugar proton is hidden under hydroxyl signals around δ 4·9.

Compound 2. Amorphous $[\alpha]_D^{25}$ −65·3° (*c* 1, EtOH). R_v 0·47 (colour: dark red). NMR. (CD_3OD) δ 1·23 *d*, *J* 5·8 Hz (3H); 1·6–2·0 *m* (2H); 2·58 *t*, *J* 7·0 Hz (2H); 3·4–4·0 *m* (8H); 3·80 *s* (3H); 4·8 *q*, *J* 3·2 and *J* 1·8 Hz (1H); 5·35 *d*, *J* 1·8 Hz (1H); 5·57 *d*, *J* 5·6 (1H); 6·58 *s* (2H); 6·92 *q*, *J* 2·0 and *J* 8·5 Hz (1H); 7·06 *d*, *J* 2·0 Hz (1H); and 7·10 *d*, *J* 8·5 Hz (1H). The anomeric sugar proton gives a doublet at δ 5·35.

Hydrolysis of **1** *and* **2**. Glycoside **1** (50 mg) in 1M H_2SO_4 (100 ml) was treated at 100° for 3 hr. The soln was extracted with EtOAc (3 × 100 ml) and from the dried and evaporated extract **3** was obtained (30 mg), and after crystallisation from aq. EtOH crystals (20 mg). Mp 137–139° R_v 0·88 (colour: dark red) $[\alpha]_D^{25}$ +5·4° (*c*, 2 EtOH) Found: C 65·9; H 6·4. Calc. for $C_{19}H_{22}O_6$; C 65·9; H 6·4%. MS: *m/e* 41 (10% of base peak), 43(11), 44(12), 55(11), 77(14), 91(12), 115(11), 124(10), 137(52), 151(12), 165(12), 269(18), 284(11), 296(19), 316(100), 317(11), 328(80), 346(M^+ 53). λ_{max}^{KBr} 1600, 1490, 1400, 1320, 1160, 1130, 1035, 1015, 940, 905, 880, 850 and 820 cm^{-1}. NMR (CD_3OD): δ 1·68 *m* (2H); 2·56 *t*, *J* 7·6 Hz (2H); 3·44 *m* (1H); 3·55 *t*, *J* 7·0 Hz (2H); 3·78 *d*, *J* 6·4 Hz (1H); 3·80 *d*, *J* 6·4 Hz (1H); 3·81 *s* (3H); 5·49 *d*, *J* 6·0 Hz (1H); 6·68 broad *s* (2H); 6·75 *d*, *J* 8·0 Hz (1H); 6·86 *q*, *J* 2·0 and 8·0 Hz (1H); 6·98 *d*, *J* 2·0 Hz (1H). Irradiating the doublet at δ 5·49 (H-2) made the multiplet centered at δ 3·44 (H-3) to collapse to a triplet, *J* 6·4 Hz, and irradiating the multiplet centered at δ 1·68 (the two β-protons in the propanol chain) made the triplets at δ 2·54 and 3·55 to collapse to two singlets. Tetraacetate of **3** (Ac_2O–Pyr): NMR ($CDCl_3$): δ 1·8–2·1 *m* (2H), 2·01 *s* (3H), 2·04 *s* (3H), 2·26 *s* (6H), 2·63 *t*, *J* 7·0 Hz (2H), 3·6–3·9 *m* (1H), 3·81 *s* (3H), 4·06 *t*, *J* 6·0 Hz (2H), 4·27 *q*, *J* 7·5 and *J* 11·0 Hz (1H), 4·45 *q*, *J* 6·0 and 11·0 Hz (1H), 5·56 *d*, *J* 6·0 Hz (1H) and 6·8–7·1 *m* (5H). After neutralization of the aq. phase ($BaCO_3$) and evaporation D-glucose (18 mg; $[\alpha]_D^{25}$ +51°, *c*, 1 in H_2O) was identified by PC in several common systems and by GLC as a TMS derivative [6]. Similarly **3** was obtained from **2** and the sugar moiety (1 mol/1 mol of **2**) proved to be L-rhamnose. ($[\alpha]_D^{25}$ +7°, *c* = 1 in H_2O).

Methylation of **1** *and* **2**. About 10 mg of **1** and **2** respectively were methylated with MeI/DMF/NaH [5]. The methyl derivatives were purified by TLC in EtOAc. MS of heptamethyl-**1**: *m/e* 45 (100% of base peak) 71(42), 73(19), 75(34), 89(35), 99(16), 101(83), 111(64), 127(21), 155(26), 187(73), 356(61), 357(13), 388(84), 389(19), 606(M^+ 17). MS of hexamethyl-**2**: *m/e* 45(53% of base peak), 55(27), 57(34), 59(75), 69(24), 71(32), 72(24), 73(16), 75(33), 83(17), 85(22), 89(29), 97(26), 99(58), 101(72), 113(18), 115(13), 117(12), 125(31), 129(30), 145(25), 157(31), 188(14), 189(93), 356(79), 357(19), 388(100), 389(23), 576(M^+ 16). Methylated **1** and **2** were, after formolysis, hydrolysis subsequent reduction and acetylation, studied by GLC–MS [3,4]. Thereby 2,3,4,6-tetra-*O*-methyl-1,5-di-*O*-acetyl-glucitol and 2,3,4-tri-*O*-methyl-1,5-di-*O*-acetyl-rhamnitol were identified from methylated **1** and **2** respectively, thus verifying the pyranoside forms of the sugars.

Formation of dihydrodehydrodiconiferyl alcohol (**4**) *from* **1** *and* **2**. Compound **1** (20 mg) in MeOH (10 ml), was treated with excess CH_2N_2 for 4 hr. Hydrolysis and extraction with EtOAc gave **4** (*ca* 10 mg), R_v 0·90 (colour: green). MS: *m/e* 41 (13% of base peak), 42(52), 77(12), 91(12), 137(35), 151(13), 165(10), 253(10), 265(12), 266(10), 283(20), 297(13), 298(13), 310(17), 311(15), 327(46), 328(11), 329(10), 330(50), 331(12), 342(100), 343(23), 360(M^+ 58), 361(15). λ_{max}^{KBr} 1605, 1515, 1495, 1460, 1350, 1320, 1270, 1210, 945, 855 and 810 cm^{-1}. Compound **4** was identical (TLC, IR and MS) with an authentic sample of dihydrodehydrodiconiferyl alcohol. Compound **4** was also obtained when compound **2** was treated in the same way.

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