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DILIGNOL GLYCOSIDES FROM NEEDLES OF *PICEA ABIES**

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Key Word Index—*Picea abies*; Pinaceae; dilignol glycosides; dihydrodehydrodiconiferyl alcohol; (−)-isolariciresinol.

Abstract—(2*R*,3*R*)-2,3-Dihydro-2-(4′-hydroxy-3′-methoxyphenyl)-3-(hydroxymethyl)-7-methoxy-5-benzofuranpropanol 4′-*O*-β-D-glucopyranoside [dihydrodehydrodiconiferyl alcohol glucoside], (2*R*,3*R*)-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, 1-(4′-hydroxy-3′-methoxyphenyl)-2-[2″-hydroxy-4″-(3-hydroxypropyl)phenoxy]-1,3-propanediol 1-*O*-β-D-glucopyranoside and 4′-*O*-β-D-xylopyranoside, 2,3-bis[(4′-hydroxy-3′-methoxyphenyl)-methyl]-1,4-butanediol 1-*O*-β-D-glucopyranoside [(−)-seco-isolariciresinol glucoside] and (1*R*,2*S*,3*S*)-1,2,3,4-tetrahydro-7-hydroxy-1-(4′-hydroxy-3′-methoxyphenyl)-6-methoxy-2,3-naphthalenedimethanol α^2-*O*-β-D-xylopyranoside [(−)-isolariciresinol xyloside] have been isolated from needles of *Picea abies* and identified.

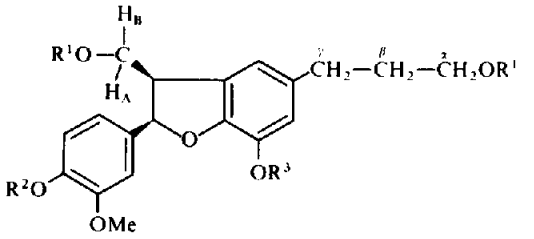
INTRODUCTION

In a previous paper on the constituents of the needles of *Pinus sylvestris* L. [1, 2], the isolation and identification of the dilignol glycosides **5–9**, among other phenolic compounds, were described. In this investigation, the dilignol glycosides from the needles of *Picea abies* have been examined. A more detailed presentation of the hydrophilic components of the acetone and aqueous acetone extracts of spruce needles will be published elsewhere.

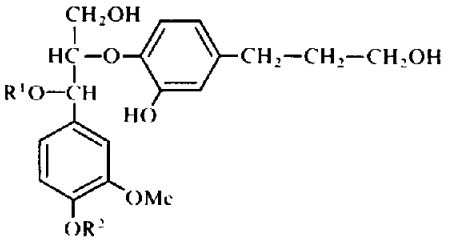
RESULTS AND DISCUSSION

A series of dilignol glycosides were isolated from the hydrophilic components of the acetone extracts of spruce needles (total amount about 0.3%). Compounds **5–9** were identified by direct comparison ([α], TLC, ^{1}H NMR, MS) with authentic samples previously isolated from *Pinus sylvestris*. Notably, a *threo–erythro* mixture of **8** but only the *threo* isomer of **7** was isolated. This agrees with the results obtained from the investigation of *P. sylvestris* [2].

The previously reported glucoside **1** ($[\alpha]_D^{22}$ −32.5°) [3] was obtained amorphous but chromatographically homogeneous. Its ^{1}H NMR spectrum showed two methoxyl groups, but was otherwise very similar to that of **5**. When it was hydrolysed with β-glucosidase, equimolar amounts of D-glucose and an amorphous aglycone ($[\alpha]_D^{22}$ 4.7°) were obtained. The aglycone was identical (NMR and MS) with an authentic sample of 2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-3-(hydroxymethyl)-7-methoxy-5-benzofuranpropanol (dihydrodehydrodiconiferyl alcohol). The glucoside was also identical with the monomethyl ether of **5**, obtained from



1 R¹ = H; R² = β-D-glucopyranoside; R³ = Me
2 R¹ = Ac; R² = β-D-glucopyranoside (Ac_4); R³ = Me
3 R¹ = R² = H; R³ = Me
4 R¹ = R² = Ac; R³ = Me
5 R¹ = R³ = H; R² = β-D-glucopyranoside
6 R¹ = R³ = H; R² = α-L-rhamnopyranoside



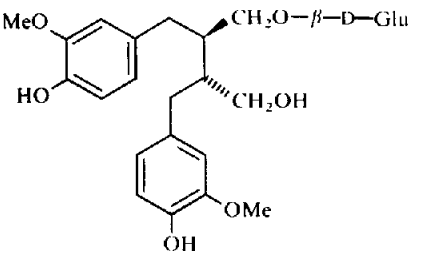
7 R¹ = β-D-glucopyranoside; R² = H
8 R¹ = H; R² = β-D-xylopyranoside

* Part 8 in the series "The Constituents of Conifer Needles". For Part 7 see Norin, T., Sundin, S. and Theander, O. (1980) *Acta Chem. Scand.* **B34**, 301.

its reaction with diazomethane. This proved that **1** was 2,3- dihydro - 2 - (4' - hydroxy - 3' - methoxyphenyl) - 3 - (hydroxymethyl) - 7 - methoxy - 5 - benzofuranpropanol 4'-*O*-β-D-glucopyranoside. The configuration of each of the compounds **1**, **3**, **5** and **6** was shown to be (2*R*,3*R*) by comparison with the 'neolignans', i.e. 2,3-dihydro-3-methyl-2-phenylbenzofurans [4, 5]. Thus, the NMR shift for H-2 was $\delta \approx 5.5$ for **1**, **3**, **5** and **6**; $\delta \approx 5.6$ for 2,3-*cis*-neolignans, and $\delta \approx 4.9$ for 2,3-*trans*-neolignans. This shift would hardly be affected by the change from methyl to hydroxymethyl at C-3. Moreover, the ORD spectrum for **3** agreed with the published ORD data for (2*R*,3*S*)-neolignans. **5** and **6** had identical aglycones which could easily be converted to **3** [1].

1 has recently been isolated from *Larix leptolepis* [6], and an isomer with glucose linked at the hydroxymethyl group at C-3 has been isolated from *Pteris vittata* [7]. It is notable that the aglycone of the latter compound has an optical rotation ($[\alpha]_D^{21}$ −8.5°) which differs markedly from that of aglycone **3**, and the NMR spectra of the acetylated aglycones are not identical, indicating that the aglycone from *P. vittata* is an isomer of **3**.

10 ($[\alpha]_D^{22}$ −55.1°) was obtained amorphous but chromatographically homogeneous. Acid hydrolysis yielded D-xylose and an aglycone having ^{1}H NMR and MS spectra identical with those of (+)-isolariciresinol, but the optical rotations were of opposite signs. The CD spectrum of the aglycone was symmetrically opposite to the spectrum of (+)-isolariciresinol, with known absolute configuration [8, 9], and also in agreement with published CD data for the 5'-methoxy derivative [10]. Hence, the aglycone was (−)-isolariciresinol (**11**). Permethylation of **10** gave the octamethyl derivative **12**. The product obtained by hydrolysis of **12** had ^{1}H NMR and MS data identical with those published for **13** [2]. The ^{1}H NMR coupling constant of 6.3 Hz shown by the anomeric proton in **10** and the detection of a 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl-pentitol by GC/MS after formolysis, hydrolysis, subsequent reduction and acetylation [11, 12] of **12** confirmed that **10** is the β-D-xylopyranoside of (−)-isolariciresinol. (+)-Isolariciresinol was reported from the resin of *Picea excelsa* [13], and an arabinoside and two glucosides of (+)-isolariciresinol were previously isolated from *Pinus sylvestris* [2]. To our knowledge, however, the (−)-form has not been reported previously, although xylosides of the 5'-methoxy derivative ($[\alpha]_D^{25}$ −53°) and of the 8,5'-dimethoxy derivative ($[\alpha]_D^{25.5}$ −67.1°) have been identified in *Cinnamosma madagascariensis* [10] and in *Enkianthus nudipes* [14] respectively.



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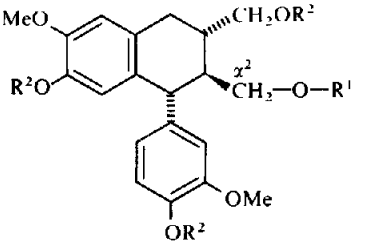

10 R¹ = β-D-xylopyranoside; R² = H
11 R¹ = R² = H
12 R¹ = β-D-xylopyranoside (OMe)₃; R² = Me
13 R¹ = H; R² = Me

EXPERIMENTAL

Fresh needles of *P. abies* (1.00 kg, dry wt 0.45 kg), collected in November, near Uppsala, Sweden, were refluxed with Me_2CO (3 l.) for 30 min. After filtration, the needles were dried, milled and extracted in an ultrasonic bath at room temp. with Me_2CO (4 × 1.0 l.) and aq. 50% Me_2CO (4 × 0.8 l.) for 8 × 40 min. The precipitating wax fraction was removed from the cooled extracts. The extracts were combined, concd to a small vol. and extracted with $CHCl_3$ (3 × 0.5 l.), EtOAc (3 × 0.5 l.) and MeCOEt (5 × 0.5 l.). From the residual aq. fraction (dry wt 76.3 g) and from the MeCOEt extract (dry wt 38.2 g), the main part of the dilignol glycosides was isolated, in a way similar to that previously described [2], by Sephadex LH-20 column chromatography (H_2O and aq. EtOH of increasing EtOH content), followed by subfractionation on silicic acid columns (230–400 mesh, $CHCl_3$–MeOH–H_2O, 40:20:1). TLC was performed on Si gel HF_{254} plates ($CHCl_3$–MeOH–H_2O). After the plates had been inspected in UV radiation, 0.1% diazotized sulfanilic acid in 10% Na_2CO_3, followed by 50% H_2SO_4, was used as spray reagent [1]. ^{1}H NMR: 100 MHz with TMS as int. standard. Data for the strongly coupled protons in **4** were obtained by ABX analysis, checked by computer simulation and adjusted if necessary [15]. MS (probe) 70 eV.

1. Amorphous (168 mg), $[\alpha]_D^{22}$ −32.5° (MeOH; *c* 2.7). ^{1}H NMR (CD_3OD): δ 1.64–1.94 (2 H, H-β), 2.60 (2 H, *t* (*br*), *J* = 7 Hz, H-γ), 3.2–3.9 (11 H, *m*), 3.80 (3 H, *s*, OMe), 3.83 (3 H, *s*, OMe), 5.54 (1 H, *d*, *J* = 5.8 Hz, H-2), 6.70 (2 H, *s* (*br*), H-4 and H-6), 6.91 (1 H, *dd*, *J* = 2.0 and 8.4 Hz, H-6'), 7.02 (1 H, *d*, *J* = 2.0 Hz, H-2'), 7.13 (1 H, *d*, *J* = 8.4 Hz, H-5'). The signal from the anomeric sugar proton, hidden under the broad hydroxyl peak, was visualized by warming the sample; δ 4.88 (1 H, *d*, *J* = 6.5 Hz).

2. Acetylation of **1** (Ac_2O–pyridine) gave an amorphous powder $[\alpha]_D^{22}$ −11.4° ($CHCl_3$; *c* 1.3). ^{1}H NMR ($CDCl_3$): δ 2.02 (9 H, *s* (*br*), 3 Ac), 2.06 (9 H, *s* (*br*), 3 Ac), 2.64 (2 H, *t* (*br*), *J* = 7 Hz, H-γ), 3.60–5.35 (12 H, *m*); 3.80 (3 H, *s*, OMe), 3.88 (3 H, *s*, OMe), 4.09 (2 H, *d*, *J* = 6.2 Hz, H-α); 5.47 (1 H, *d*, *J* = 7.2 Hz, H-2), 6.60–7.40 (5 H, *m*). The signals from the β-CH_2 protons were hidden under Ac signals around δ 2.

3. **1** (53 mg) in H_2O (5 ml) was treated with β-emulsin (5 mg) overnight at room temp. The mixture was extracted with EtOAc (3 × 15 ml), followed by evapn and column chromatography on Si gel. **3** (29 mg) was obtained as an amorphous powder, $[\alpha]_D^{22}$ 4.7° (Me_2CO; *c* 2.0). ^{1}H NMR (CD_3OD): δ 1.82–1.98 (2 H, *m*, H-β), 2.62 (2 H, *t* (*br*), *J* = 7 Hz, H-γ), 3.4–4.0 (5 H, *m*), 3.90 (3 H, *s*, OMe), 3.94 (3 H, *s*, OMe), 4.83 (*s* (*br*), OH), 5.48 (1 H, *d*, *J* = 5.8 Hz, H-2), 6.68–6.98 (5 H, *m*); MS *m/z* (rel. int.): 360 (59, M^+), 342 (100), 330 (49), 327 (32), 311 (10), 310 (13), 283 (11), 137 (13); ORD (*c* 50 mg/100 ml, MeOH, 215–400 nm): $[\phi]_{299}$ 1700, $[\phi]_{293}$ 0, $[\phi]_{284}$ −700, $[\phi]_{266}$ 0, $[\phi]_{251}$ 1900, $[\phi]_{245}$ 0, $[\phi]_{234}$

-6000, $[\phi]_{233}$ 0, $[\phi]_{217}$ 6400. M^+, obs. 360.155, calc. for $C_{20}H_{24}O_6$: 360.157. D-Glucose was identified in the aq. layer ($[\alpha]$, TLC, GLC).

4. Acetylation of **3** (Ac_2O–pyridine) gave an amorphous powder, $[\alpha]_D^{22}$ 5.5° ($CHCl_3$; *c* 0.7). ^1H NMR ($CDCl_3$): δ 2.05 (3 H, *s*, OAc), 2.07 (3 H, *s*, OAc), 2.30 (3 H, *s*, OAc), 2.63 (2 H, *t* (*br*), J = 7 Hz, H-γ), 3.81 (3 H, *s*, OMe), 3.89 (3 H, *s*, OMe), 4.09 (2 H, *t*, J = 6.6 Hz, H-α), 4.31 (1 H, *dd*, J_{AB} = 10.9 Hz, $J_{B,3}$ 5.6 Hz, H_B), 4.47 (1 H, *dd*, $J_{A,3}$ 7.1 Hz, H_A), 5.51 (1 H, *d*, J = 7.2 Hz, H-2), 6.6–7.0 (5 H, *m*). The signals from the protons on the β-C are hidden under the signals from the Ac protons around δ 2.0.

5 (467 mg), **6** (115 mg), **7** (256 mg), **8** (84 mg) and **9** (35 mg) were identical in all respects (TLC, $[\alpha]$, NMR, MS) with authentic samples.

Methylation of **5**. **5** (23 mg) was treated with excess ethereal CH_2N_2 for 2 hr, and a compound (18 mg) identical (NMR, $[\alpha]$, TLC) with **1** was obtained.

10. Amorphous (45 mg), $[\alpha]_D^{22}$ −55.1° (MeOH; *c* 0.9). ^1H NMR (CD_3OD): δ 1.8–2.0 (2 H, *m*, H-2 and H-3), 2.55–3.95 (12 H, *m*), 3.78 (3 H, *s*, OMe), 3.79 (3 H, *s*, OMe), 4.00 (1 H, *d*, J = 6.3 Hz, H-1_{xyl}), 6.18 (1 H, *s*), 6.40–6.85 (4 H, *m*).

11. **10** (14 mg) was treated with 1 M H_2SO_4 overnight at room temp. The mixture was extracted with EtOAc (3 × 10 ml) and the extract was evapd and chromatographed on a Si gel column to give **11** (4 mg), $[\alpha]_D^{22}$ −48.6° (Me_2CO; *c* 0.4). ^1H NMR and MS spectra identical with those of authentic (+)-isolariciresinol; CD (*c* 50 mg/100 ml MeOH, 190–400 nm): $[\theta]_{390}$ 0, $[\theta]_{363}$ −25, $[\theta]_{350}$ 0, $[\theta]_{335}$ 90, $[\theta]_{323}$ 0, $[\theta]_{311}$ −110, $[\theta]_{306}$ 0, $[\theta]_{293}$ 21 900, $[\theta]_{284}$ 0, $[\theta]_{276}$ −14 100, $[\theta]_{253}$ −860, $[\theta]_{239}$ −25 900, $[\theta]_{227}$ −4600, $[\theta]_{215}$ −41 800, $[\theta]_{211}$ 0, $[\theta]_{205}$ 108 000, $[\theta]_{199}$ 0, CD for authentic (+)-isolariciresinol is symmetrically opposite. D-Xylose was identified in the aq. layer ($[\alpha]$, TLC, GLC).

12. Methylation (MeI/Me_2SO, NaH) of **10** (11.5 mg) yielded the permethylated product (11.4 mg, amorphous), $[\alpha]_D^{22}$ −86° ($CHCl_3$; *c* 0.3). ^1H NMR ($CDCl_3$): δ 1.6–4.0 (14 H, *m*), 3.36 (3 H, *s*, OMe), 3.48 (3 H, *s*, OMe), 3.55 (3 H, *s*, OMe), 3.59 (3 H, *s*, OMe), 3.61 (3 H, *s*, OMe), 3.82 (3 H, *s*, OMe), 3.85 (3 H, *s*, OMe), 3.87 (3 H, *s*, OMe), 4.11 (1 H, *d*, J = 6.3 Hz, H-1_{xyl}), 6.26 (1 H, *s*), 6.5–6.8 (4 H, *m*); MS *m/z* (rel. int.): 576 (7, M^+), 402 (17), 401 (26), 383 (100), 370 (12), 353 (22), 339 (31), 269 (18), 151 (48), 116 (20). The products obtained from **12** after formolysis, hydrolysis, subsequent reduction and acetylation were studied by GC/MS; 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylpenitol was thereby identified, thus confirming the pyranoside form of xylose.

13. **12** (11 mg) was methanolysed (1% HCl in MeOH) on a water-bath for 3 hr. Extraction with Et_2O and purification on a Si gel column (petrol, bp 40–60°–EtOAc, 1:1) yielded 2 mg of **13**, $[\alpha]_D^{22}$ −25° ($CHCl_3$; *c* 0.1). ^1H NMR and MS identical with those of authentic samples.

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