Acta Chemica Scandinavica B 39 (1985) 241–248

# The Constituents of Conifer Needles. Dilignol Glycosides from *Pinus massoniana* Lamb.*

LENNART N. LUNDGREN, ZHAOBANG SHEN** and OLOF THEANDER

Department of Chemistry and Molecular Biology, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden

**Seventeen dilignol glycosides and two arylglycerols have been isolated and identified from *Pinus massoniana* Lamb. needles. They consisted of two α-L-rhamnopyranosides of 2,3-dihydro-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-7-methoxy-5-benzofuranpropanol; two α-L-rhamnopyranosides and a β-D-glucopyranoside of 2,3-dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol; an α-L-rhamnopyranoside, two β-D-glucopyranosides and a β-D-xylopyranoside of 1-(4′-hydroxy-3′-methoxyphenyl)-2-[2″-hydroxy-4″-(3-hydroxypropyl)phenoxy]-1,3-propanediol; an α-L-rhamnopyranoside and a β-D-glucopyranoside of 1-(4′-hydroxy-3′-methoxyphenyl)-2-[4″-(3-hydroxypropyl)-2″-methoxyphenoxy]-1,3-propanediol; a β-D-xylopyranoside, a β-D-glucopyranoside and an α-L-arabinofuranoside of (+)-isolariciresinol; a β-D-glucopyranoside and a β-D-xylopyranoside of (−)-seco-isolariciresinol and a β-D-glucopyranoside of (+)-pinoresinol. The two arylglycerols were 1-(4-hydroxyphenyl)-1,2,3-propanetriol and 1-(4-hydroxy-3-methoxyphenyl)-1,2,3-propanetriol.**

**In this communication, we describe the identification of a series of dilignol glycosides and two arylglycerols, isolated together with some flavanoids[1] and a new lignan,[2] from *Pinus massoniana* Lamb.**

## RESULTS AND DISCUSSION

By column chromatography on Sephadex LH-20 and Si-gel using different solvent systems, seventeen dilignol glycosides and two arylglycerols were isolated from *Pinus massoniana* Lamb. needles. These glycosides may be grouped as glycosides of 2,3-dihydro-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-7-methoxy-5-benzofuranpropanol (*1, 2*); 2,3-dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol (*4–6*); 1-(4′-hydroxy-3′-methoxyphenyl)-2-[2″-hydroxy-4″-(3-hydroxypropyl)phenoxy]-1,3-propanediol (*8–11*), 1-(4-hydroxy-3-methoxyphenyl)-2-[4-(3-hydroxypropyl)-2-methoxyphenoxy]-1,3-propanediol (*13, 14*); (+)-isolariciresinol (*16–18*); (−)-secoisolariciresinol (*20, 21*) and (+)-pinoresinol (*23*).

Eight of the glycosides (*5, 6, 8–10, 17, 18, 20*) have previously been isolated from *Pinus sylvestris* (all the eight glycosides)[3,4] and *Picea abies* (*5, 6, 9, 10, 20*)[5] and they were identified by the direct comparison ($^1$H NMR, TLC, $[\alpha]_D$) with authentic samples.

* Part 12 in the series The Constituents of Conifer Needles; Part 11, see Ref. 2.

** Present address: Research Institute of Chemical Processing and Utilization of Forest Products, Chinese Academy of Forestry, Shao Shan Road, Nanjing, The People's Republic of China.

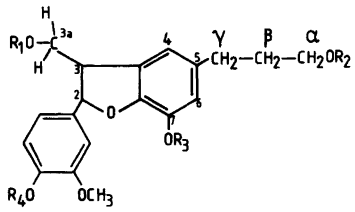
0302-4369/85 $2.50

Compounds *1* and *2* proved to be rhamnosides of the same aglycone and differed only in the position of the linkage between the sugar moiety and the aglycone. On acid hydrolysis, both compounds gave L-rhamnose and the aglycone *3*, identical with an authentic sample of dihydrodehydrodiconiferyl alcohol ($^1$H NMR, TLC, $[\alpha]_D$). The position of the sugar linkage was determined by the comparison of the $^1$H NMR spectra of the pentaacetate of *1* and *2* with that of the triacetate of *3*. The $^1$H NMR of acetylated *3* showed the presence of one aryl and two aliphatic acetoxyl groups, as well as two methylene signals, shifted downfield as a triplet at $\delta$ 4.09 and two quartets at $\delta$ 4.31 and 4.37 owing to the acetylation.[5] The $^1$H NMR of the pentaacetate of *1* showed one aryl and four aliphatic acetoxyl groups, indicating that the sugar moiety was attached to one of the aliphatic hydroxyl groups and the presence of only one methylene signal at $\delta$ 4.09 was consistant with a linkage at the 3a-hydroxyl group. Therefore compound *1* was 2,3-dihydro-2-(4′-hydroxy-3′-methoxyphenyl)-3a-$\alpha$-L-rhamnopyranosyloxymethyl-7-methoxy-5-benzofuranpropanol.

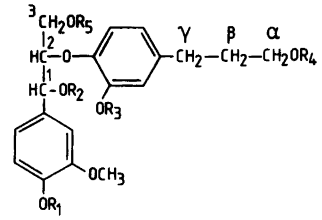
The $^1$H NMR of acetylated *2*, unlike that of *1*, showed, inter alia, five aliphatic acetoxyl groups and two methylene signals, clearly indicating that the rhamnose moiety was linked to the aromatic hydroxyl group in compound *2*. Furthermore compound *2* was also identical ($^1$H NMR, TLC, $[\alpha]_D$) with the 7-*O*-methyl ether of compound *5*, obtained by methylation with diazomethane. Hence compound *2* was 2,3-dihydro-2-(4′-$\alpha$-L-rhamnopyranosyloxy-3′-methoxyphenyl)-3-hydroxymethyl-7-methoxy-5-benzofuranpropanol.

Compound *4*, identified as 2,3-dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3a-$\alpha$-L-rhamnopyranosyloxymethyl-5-benzofuranpropanol has been found in the inner bark of *Larix leptolepis* Gord.[6] The $[\alpha]_D$, $^1$H NMR of *4* and its peracetate, as well as the MS of its hexamethyl ether were consistent with the literature data.[6] The aglycone of compound *4* was identical with authentic *7*, available in this laboratory ($^1$H NMR, TLC, $[\alpha]_D$).

Compound *11* was isolated as a mixture of its *threo*- and *erythro*-isomers in the ratio of three to one. The $^1$H NMR of *11* was similar to those of compounds *8*–*10*. On enzymatic hydrolysis with cellulase *11* gave glucose and an aglycone, identical with authentic *12*. The $^1$H NMR of the octaacetate of *11* showed two aromatic and six aliphatic acetoxyl groups. Furthermore, the signals of the benzylic proton and the methylene protons (H-$\alpha$) shifted downfield owing to the acetylation. Thus, the glucose is presumably attached to the 3-hydroxyl group. The sugar moiety was identical by GLC but owing to the small amount available its absolute configuration was not determined. The MS of the octamethyl ether of *11* further confirmed the structure with the peak m/e 639 (M+1) and the fragments *m/e* 442



*1* $R_1$ = α-L-rhamnosyl $R_2$=$R_4$=H $R_3$=Me
*2* $R_1$=$R_2$=H $R_3$=Me $R_4$= α-L-rhamnosyl
*3* $R_1$=$R_2$=$R_4$=H $R_3$=Me
*4* $R_1$= α-L-rhamnosyl $R_2$=$R_3$=$R_4$=H
*5* $R_1$=$R_2$=$R_3$=H $R_4$= α-L-rhamnosyl
*6* $R_1$=$R_2$=$R_3$=H $R_4$= β-D-glucosyl
*7* $R_1$=$R_2$=$R_3$=$R_4$=H



*8* $R_1$=$R_2$=$R_3$=$R_5$=H $R_4$= α-L-rhamnosyl
*9* $R_1$=$R_3$=$R_4$=$R_5$=H $R_2$= β-D-glucosyl
*10* $R_2$=$R_3$=$R_4$=$R_5$=H $R_1$= β-D-xylosyl
*11* $R_1$=$R_2$=$R_3$=$R_4$=H $R_5$= β-D-glucosyl
*12* $R_1$=$R_2$=$R_3$=$R_4$=$R_5$=H
*13* $R_1$=$R_2$=$R_5$=H $R_3$=Me $R_4$= α-L-rhamnosyl
*14* $R_1$=$R_2$=$R_5$=H $R_3$=Me $R_4$= β-D-glucosyl
*15* $R_1$=$R_2$=$R_4$=$R_5$=H $R_3$=Me

and 181, characteristic of the sugar linkage to the 3-hydroxyl group.[4] Thus compound *11* is a new glycoside of the dilignol *12*, namely its 3-*O*-glucoside.

Compounds *13* and *14* were shown to be different glycosides of the same aglycone *15*. Both glycosides *13* and *14*, as well as the aglycone *15* gave a bright orange colour with diazotized sulfanilic acid.[3] Compound *13* was isolated as a mixture of its *threo*- and *erythro*-isomers, which could be distinguished by their benzylic proton signals (as two doublets around $\delta$ 4.9) in the $^1$H NMR. The ratio of the *threo*- and *erythro*-isomers was different in the two needle samples (see Experimental), 1:2 for sample A and 3:1 for sample B. This is presumably due to the different seasons of collecting the samples.

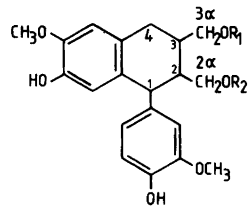
The $^1$H NMR spectrum of *13* was very similar to that of *8*, except for the presence of two methoxyl groups. On enzymatic hydrolysis with pectinase, which had been shown to have some $\alpha$-rhamnosidase activity,[7,8] L-rhamnose and an aglycone *15* were obtained. The $^1$H NMR of *15* showed the presence of two methoxyl groups, one benzylic proton at $\delta$ 4.83 and six protons, characteristic of the phenyl propanol side chain. Acetylation of *15* yielded the tetraacetate with three aliphatic and one aromatic acetoxyl groups. The $^1$H NMR of *15* and its peracetate were similar to that of compound *12*.

In order to confirm the structure of *15*, an authentic sample was prepared from compound *10*. *10* was methylated with diazomethane and then hydrolysed with cellulase. The aglycone was found to be identical with compound *15* ($^1$H NMR, TLC). Therefore, compound *15* is 1-(4′-hydroxy-3′-methoxyphenyl)-2-[4″-(3-hydroxypropyl)-2″-methoxyphenoxy]-1,3-propanediol.

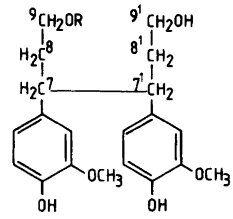
The $^1$H NMR of the hexaacetate of *13* showed five aliphatic and one aromatic acetoxyl groups but the $\alpha$-$CH_2OAc$ signal (t. $\delta$ 4.07) was absent. This indicated that the rhamnosyl group must be attached to the $\alpha$-carbinol group in *13*. Furthermore, the MS of the hexamethyl ether of *13* was found to be identical with the MS of the heptamethyl ether of compound *8*. *13* may thus be assigned the structure 1-(4′-hydroxy-3′-methoxyphenyl)-2-[4″-(3-$\alpha$-L-rhamnosyloxypropyl)-2″-methoxyphenoxy]-1,3-propanediol. A rhamnoside has been isolated from the *Pinus contorta* needles,[9] which is probably identical with compound *13*, but its structure was not confirmed.

Compound *14* was isolated as its pure *erythro*-isomer, as shown by its $^1$H NMR. After hydrolysis with cellulase, *14* afforded D-glucose and the aglycone, identical with the compound *15* ($^1$H NMR, TLC). The aglycone of *14* also appeared as its *erythro*-isomer. In the $^1$H NMR spectra of the heptaacetate of 14, six aliphatic groups and one aromatic acetoxyl group were evident. No proton signal around $\delta$ 4.07 for a $\alpha$-$CH_2OAc$ was found. This evidence indicated that the sugar is presumably attached at the $\delta$-carbinol group and this was confirmed by the presence of the characteristic fragments m/e 238 and 181 in the MS of the heptamethyl ether of *14*.[4] Hence, compound *14* was assigned the structure 1-(4′-hydroxy-3′-methoxyphenyl)-2-[4″-(3-$\beta$-D-glucopyranosyloxypropyl)-2″-methoxyphenoxy)]-1,3-propanediol.

Compound *16* crystallized as colourless needles from MeOH. The $^1$H NMR of *16* and the MS of its hexamethyl ether were found to be identical with the authentic sample of (−)isolariciresinol 2$\alpha$-*O*-$\beta$-D-xyloside, previously isolated from *Picea abies* needles.[5] The m.p., $[\alpha]_D$ of *16* and the $^1$H NMR of its hexaacetate were identical with the literature data for (+) isolariciresinol 2$\alpha$-*O*-$\beta$-D-xyloside, isolated from the woody part of *Schizandra nigra* Max.[10] The aglycone, obtained after enymatic hydrolysis, was identical with the authentic sample of (+) isolariciresinol ($^1$H NMR, TLC, $[\alpha]_D$). Furthermore, the trimethyl ether of (+)-isolariciresinol, obtained by methylation of *16* and subsequent methanolysis was



*16* $R_1$ = H $R_2$ = β-D-xylosyl
*17* $R_2$ = H $R_1$ = β-D-glucosyl
*18* $R_1$ = H $R_2$ = α-L-arabinosyl
*19* $R_1$ = $R_2$ = H



*20* R = β-D-glucosyl
*21* R = β-D-xylosyl
*22* R = H

identical to an authentic sample of 3α,7,4′-tri-O-methyl-(+)-isolariciresinol, available in this laboratory. Compound *16* was thus (+) isolariciresinol 2α-*O*-β-D-xylopyranoside.

Enzymatic hydrolysis of the amorphous xyloside *21* with cellulase, gave D-xylose and the aglycone *22*, identical with an authentic sample of (−)-secoisolariciresinol ($^1$H NMR, TLC, $[\alpha]_D$). The $^1$H NMR of the hexaaxetate of *21* revealed two aromatic and four aliphatic acetoxyl groups, indicating that the sugar moiety was linked to one of the equivalent aliphatic hydroxyl groups. The MS of its hexamethyl ether showed the molecular ion at m/e 576 ($M^+$) and an ion at 402 ($M^+$-trimethylxylosyl+H], *21* may therefore be assigned the structure, (−)-secoisolariciresinol 9-*O*-β-D-xylopyranoside.

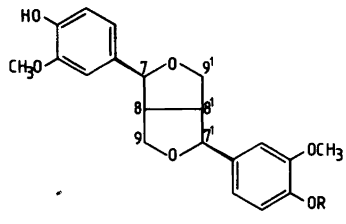
The structure of (+)-pinoresinol-4′-*O*-β-D-glucopyranooside, *23*, was confirmed by enzymatic hydrolysis with cellulase to yield D-glucose and an aglycone *24*, identical with the authentic sample of (+)-pinoresinol ($^1$H NMR, TLC, $[\alpha]_D$). The $^1$H NMR of the pentaacetate of *23* revealed four aliphatic and one aromatic acetoxyl groups, in accordance with the proposed structure. This glucoside has been isolated from the fruit of *Forsythia suspena*[11] and from the bark of *Ligustrum japonicum.*[12]

Apart from the above mentioned glycosides, two arylglycerols, *25* ($[\alpha]_D^{22}$ +3.4°) and *26* ($[\alpha]_D^{22}$ −2.3°) were also isolated in their threo-forms. The optical rotations of *25* and *26* were markedly different from those of synthetic D-*threo*-compounds ($[\alpha]_D^{22.3}$ −26.1° and ($[\alpha]_D^{23}$ −33.8°)[13] and implied that they were mixtures of their D- and L-forms.

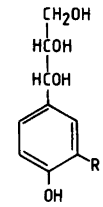
Compounds *1, 2, 11, 13–15* and *21* do not appear to have been reported by other authors. Compounds *4, 16* and *23* were found for the first time in *Pinus* needles.

## EXPERIMENTAL

The general procedure and the instruments used were as described in the previous paper.[1] Acetylation was carried out with acetic anhydride-pyridine, and permethylation with



*23* R = β-D-glucosyl
*24* R = H



*25* R = H
*26* R = OMe

MeI/DMF and NaH. Details for the hydrolysis procedures were as reported previously.[3] Compounds *1, 2, 4* were hydrolysed with 1 M $H_2SO_4$, compound *13*– with pectinase (Sigma, No. P-4625, from *Aspergillus niger*) and compounds *11, 14, 16, 21* and *23* – with crude enzyme (cellulase, practical grade, Sigma) in water at room temperature for 1–3 d. The sugar was identified by PC and GLC after trimethylsilylation. The glycosidic linkage was determined by the chemical shift and coupling constant of the anomeric proton in the $^1$H NMR of the glycoside. The absolute configuration of the sugar was determined by measuring the optical rotation (for compound *13*) and by calculating the difference in the molecular rotation between the glycoside and the aglycone, according to the Hudson-Klyne rule (for the other glycosides except *11*, which was a mixture of diastereomers). The homogeneity of isolated compounds was checked by TLC in several systems.[3]

## Isolation

*Experiment A.* The dilignol glycosides were isolated from EtOAc and water fractions of the same needle sample (sample A) as described in a previous paper.[1] Compounds *5* (130 mg), *8* (18 mg), *9* (10 mg), *11* (15 mg), *13* (36 mg), *14* (21 mg), *16* (37 mg), *17* (40 mg), *20* (28 mg), *23* (10 mg), *25* (20 mg) and *26* (18 mg) were isolated, chromatographically homogeneous, from this sample.

*Experiment B.* Another *Pinus massoniana* Lamb. needle sample (sample B) was used in this experiment. Sample B was collected in October at the arboretum of the Nanjing Technological College of Forestry in China. The air-dried needles (1300 g, water content 7.9 %) were treated and extracted in the same way as for sample A[1]. The dilignol glycosides were isolated from the EtOAc fraction (dry weight 8.4 g) and the MeCOEt fraction (dry weight 14 g) of the acetone–water extract by chromatography on Sephadex LH-20 and Si-gel columns. Compounds *1* (65 mg), *2* (230 mg), *4* (120 mg), *5* (600 mg), *6* (27 mg), *8* (130 mg), *9* (80 mg), *10* (70 mg), *12* (62 mg), *13* (45 mg), *16* (150 mg), *17* (50 mg), *18* (170 mg), *21* (38 mg) and *23* (18 mg) were obtained from sample B. Most of the xylosides and rhamnosides were isolated from the EtOAc fractions whereas the glucosides remained in the MeCOEt fraction (for sample B) and the water fraction (for sample A).

Compound *1*, amorphous, $[\alpha]_D^{22}$ −9.8° (MeOH; c 1.0). $^1$H NMR ($CD_3OD$): $\delta$ 1.24 (3 H, d, $J$=5.7 Hz, rhamnose 5-Me), 1.7–2.0 (2 H, m, $\beta$-$CH_2$-), 2.63 (2 H, br.t., $J$=7.5 Hz, $\gamma$-$CH_2$-), 3.4–3.9 (9 H, m), 3.82, 3.85 (6 H, 2×S 2×Ar–OMe), 4.73 (1 H, d, $J$=1.6 Hz, rhamnose 1-H), 5.47 (1 H, d, $J$=6.0 Hz, H-2), 6.70–7.0 (5 H, m, aromatic protons).

Acetylation of *1* (10 mg) gave the pentaacetate (8 mg). $^1$H NMR ($CDCl_3$): $\delta$ 1.21 (3 H, d, $J$=6.0 Hz, rhamnose 5-Me), 1.8–2.1 (2 H, m, $\beta$-$CH_2$-), 1.99, 2.05, 2.06, 2.15 (12 H, 4×S, 4×-OAc), 2.30 (3 H, S, Ar–OAc), 2.64 (2 H, br.t. $J$=7.5 Hz, $\gamma$-$CH_2$-), 3.6–4.1 (4 H, m), 3.84, 3.91 (6 H, 2×S, 2×Ar–OMe), 4.10 (2 H, t, $J$=7.0 Hz, $\alpha$-$CH_2$–OAc) 4.81 (1 H, br.s. rhamnose 1-H), 4.9–5.4 (3 H, m, rhamnose 2, 3, 4-H), 5.59 (1 H, d, $J$=4.0 Hz, H-2) 6.65 (2 H, br.s. aromatic protons), 7.01 (3 H, s, aromatic protons).

Methylation of *1* (8 mg) gave a pentamethyl ether (6 mg). MS (probe, 70 eV), *m/z* (rel. int.): 576 ($M^+$, 2), 388 (1), 370 (20), 358 (22), 221 (21), 189 (36), 151 (69), 101 (82), 88 (48), 75 (60), 59 (78), 45 (100).

Compound *2*, amorphous, $[\alpha]_D^{22}$ −55° (MeOH; c 0.91). $^1$H NMR ($CD_3OD$): $\delta$ 1.22 (3 H, d, $J$=6.0 Hz, rhamnose 5-Me), 1.7–2.0 (2 H, m, $\beta$-$CH_2$-), 2.63 (2 H, t, $J$=7.5 Hz, $\gamma$-$CH_2$-), 3.4–4.1 (9H, m), 3.79, 3.85 (6H, 2×S, 2×Ar–OMe), 5.34 (1 H, d, $J$=1.7 Hz, rhamnose 1-H), 5.55 (1 H, d, $J$=6.0 Hz, H-2), 6.72 (2 H, br.s. aromatic protons), 6.8–7.2 (3 H, m, aromatic protons).

Acetylation of *2* (10 mg) yielded the pentaacetate (8 mg). $^1$H NMR ($CDCl_3$): $\delta$ 1.20 (3 H, d, $J$=6.0 Hz, rhamnose 5-Me), 1.8–2.1 (2 H, m, $\beta$-$CH_2$-), 2.02, 2.03 (6 H, 2×S, 2×-OAc), 2.07 (6 H, s, 2×-OAc), 2.18 (3 H, s, -OAc), 2.64 (2 H, br.t., $J$=7.5 Hz, $\gamma$-$CH_2$-), 3.6–4.1 (2H, m), 3.83, 3.89 (6 H, 2×S, 2×Ar–OMe), 4.10 (2 H, t, $J$=6.5 Hz, $\alpha$-$CH_2$-OAc), 4.1–4.5

(2 H, m, H-3a), 5.0–5.7 (5 H, m), 6.65 (2 H, br.s., aromatic protons), 6.8–7.1 (3 H, m, aromatic protons).

Methylation of *2* (17 mg) gave the pentamethyl ether (12 mg). MS (probe, 70 eV), *m/z* (rel. int.): 576 ($M^+$, 1), 388 (5), 356 (7), 189 (37), 157 (18), 145 (17), 101 (68), 89 (29), 75 (48), 59 (78), 45 (100).

Methylation of *5* (32 mg) with excess ethereal $CH_2N_2$ for 2 h gave a compound (20 mg), identical with compound *2* ($^1$H NMR, $[\alpha]_D$, TLC).

Compound *11,* mixture of *threo-* and *erythro*-isomers in ratio of 3:1. $^1$H NMR ($CD_3OD$): $\delta$ 1.6–2.0 (2 H, m, $\beta$-$CH_2$-), 2.55 (2 H, br.t., $J$=7.5 Hz, $\gamma$-$CH_2$-) 3.1–4.1 (8 H, m), 3.54 (2 H, t, $J$=6.0 Hz, $\alpha$-$CH_2$-), 3.81 (3 H, s, -OMe), 4.24 (1 H, d, $J$=7.0 Hz, glucose 1-H), 4.1–4.4 (1 H, m, H-2), 6.6–7.0 (6 H, m, aromatic protons). The benzylic proton (H-1) signal was hidden under the broad hydroxyl peak and appeared at $\delta$ 4.83 ($\frac{1}{4}$ H, d, $J$=5.0 Hz) and 4.90 ($\frac{3}{4}$ H, d, $J$=6.2 Hz) while recording the spectrum at 50 °C.

Acetylation of compound *11* (5 mg) gave the octaacetate (3 mg). $^1$H NMR ($CDCl_3$): $\delta$ 1.8–2.3 (2 H, m, $\beta$-$CH_2$-), 1.90–2.10 (18H, m, 6×-OAc), 2.18 ($\frac{1}{4}$×3 H, s, Ar–OAc), 2.27 ($\frac{3}{4}$×3H, s, Ar–OAc), 2.30 (3 H, s, Ar–OAc), 2.61 (2 H, br.t. $J$=7.5 Hz, $\gamma$-$CH_2$-), 3.5–4.3 (5H, m), 3.83 (3 H, s, -OMe), 4.07 (2 H, t, $\alpha$-$CH_2$-), 4.4–4.8 (1 H, m, H-2), 4.9–5.2 (4 H, m), 6.06 ($\frac{1}{4}$ H, d, $J$=7.5 Hz, H-1), 6.08 ($\frac{3}{4}$ H, d,. $J$=7.0 Hz, H-1), 6.8–7.1 (6H, m, aromatic protons).

Methylation of compound *11* gave the octamethyl ether. MS (probe 30 eV), *m/z* (rel. int.): 639 (M+1, 0.1), 442 (1), 404 (4), 313 (10), 222 (15), 219 (8), 218 (12), 207 (13), 196 (10), 187 (65), 182 (8), 181 (100), 163 (15), 155 (15), 151 (30), 127 (13), 111 (50), 101 (40), 88 (29), 75 (29), 71 (19), 45 (23)

Compound *13a,* amorphous, a mixture of *threo-* and *erythro*-isomers in ratio 1:2, isolated from sample A. $^1$H NMR ($CD_3OD$): $\delta$ 1.24 (3 H, d, $J$=6.0 Hz, rhamnose 5-Me), 1.7–2.0 (2 H, m, $\beta$-$CH_2$-), 2.63 (2 H, br.t., $J$=7.5 Hz, $\gamma$-$CH_2$-), 3.4–3.9 (8 H, m), 3.79 (3×$\frac{2}{3}$ H, s, -OMe), 3.80 (3×$\frac{2}{3}$ H, s, -OMe), 3.82 (3×$\frac{1}{3}$ H, s, -OMe), 3.85 (3×$\frac{1}{3}$ H, s, -OMe), 4.0–4.2 (1 H, m, H-2), 4.64 (1H, d, $J$=1.7 Hz, rhamnose 1-H), 6.5–7.1 (6 H, m, aromatic protons). The proton signal of H-1 was hidden under the broad hydroxyl peak and appeared as two doublets at $\delta$ 4.83 (1×$\frac{2}{3}$ H, d, $J$=5.7 Hz) and 4.87 (1×$\frac{1}{3}$ H, d, $J$=6.0 Hz), when recorded at 40 °C.

Acetylation of *13a* (8 mg) yielded the hexaacetate (5 mg). $^1$H NMR ($CDCl_3$): $\delta$ 1.21 (3 H, d, $J$=6.0 Hz, rhamnose 5-Me), 1.7–2.1 (2 H, m, $\beta$-$HC_2$-), 2.0, 2.06, 2.16 (9 H, 3×s, rhamnose 3×-OAc), 2.0 (3×$\frac{2}{3}$ H, s, -OAc), 2.03 (3×$\frac{1}{3}$ H, s, -OAc), 2.06 (3×$\frac{2}{3}$ H, s, -OAc), 2.10 (3×$\frac{1}{3}$ H, s, -OAc), 2.30 (3 H, s, Ar–OAc), 2.64 (2 H, br.t., $J$=7.5 Hz, $\gamma$-$CH_2$-), 3.5–4.5 (5 H, m), 3.78 (3×$\frac{2}{3}$ H, s, -OMe), 3.81 (3 H, s, -OMe), 3.82 (3×$\frac{1}{3}$ H, s, -OMe), 4.5–4.8 (1 H, m, H 2), 4.71 (1 H, br.s, rhamnose 1-H), 5.06 (1 H, t, $J$=9.0 Hz, rhamnose 4-H), 5.2–5.4 (2 H, m, rhamnose 2,3-H), 6.07 (1×$\frac{2}{3}$ H, d, $J$=5.0 Hz, H-1), 6.12 (1×$\frac{1}{3}$ H, d, $J$=6.0 Hz, H-1), 6.7–7.1 (6 H, m, aromatic protons).

Methylation of *13a* gave the hexamethyl ether. MS (probe, 30 eV), *m/z* (rel. int.): 608 ($M^+$, 0.7), 370 (22), 358 (20), 238 (7), 207 (7), 181 (100), 151 (12).

Compound *13b,* amorphous, a mixture of *threo-* and *erythro-* isomers in ratio 3:1, isolated from the EtOAc and MeCOEt fractions of sample B. Its $^1$H NMR ($CD_3OD$) and the $^1$H NMR of its hexaacetate differed from those of *13a* only in the ratio of signals for *threo* and *erythro-* isomers.

Compound *14,* amorphous, $[\alpha]_D^{22}$ −16.5° (MeOH, c 0.4), isolated from the water fraction of sample A as a pure *erythro*-isomer. $^1$H NMR ($CD_3OD$): $\delta$ 1.7–2.1 (2 H, m, $\beta$-$CH_2$-), 2.65 (2 H, br.t., $J$=7.5 Hz, $\gamma$-$CH_2$-), 3.2–4.1 (10 H, m), 3.78, 3.80 (6 H, 2×s, 2×-OMe), 4.25 (1 H, d, $J$=7.0 Hz, glucose 1-H), 4.2–4.4 (1 H, m, H-2), 6.5–7.1 (6 H, m, aromatic protons). The benzylic proton signal was hidden under the broad hydroxyl peak but could be detected by warming the sample to 40 °C, $\delta$ 4.83 (1 H, d, $J$=5.8 Hz).

Acetylation of *14* (5 mg) gave the heptaacetate (3 mg). $^1$H NMR ($CDCl_3$): $\delta$ 1.7–2.1 (2 H, m, $\beta$-$CH_2$-), 2.01, 2.05, 2.07, 2.10 (12 H, 4×s, 4×-OAc), 2.59 (2 H, br.t., $J$=7.0 Hz, $\gamma$-$CH_2$-), 3.4–4.0 (3 H, m), 3.76, 3.81 (6 H, 2×s, 2×-OMe), 4.1–4.6 (6 H, m), 4.7–5.3 (3H, m), 6.07 (1 H, d, $J$=5.0 Hz, H-1), 6.5–7.1 (6 H, m, aromatic protons).

Methylation of *14* gave the heptamethyl ether. MS (probe 30 eV), *m/z* (rel. int.): 639 (M+1, 0.1), 404 (3), 238 (8), 207 (7), 187 (6), 182 (10), 181 (100), 151 (19), 111 (10), 101 (12), 88 (11), 75 (9), 45 (10).

Compound *15*. The *erythro*-isomer of *15* was obtained after hydrolysis of *14*, $[\alpha]_D^{22}$ −4° (MeOH; c 0.2). $^1$H NMR ($CD_3OD$): $\delta$ 1.5–2.0 (2H, m, $\beta$-$CH_2$-), 2.60 (2 H, br.t., $J$=7.0 Hz, $\gamma$-$CH_2$-), 3.54 (2 H, t, $J$=6.5 Hz, $\alpha$-$CH_2$-), 3,4–3.9 (2 H, m), 3.78; 3.80 (6 H, 2×s, 2×-OMe), 4.1–4.4 (1 H, m, H-2), 6.5–7.0 (6 H, m, aromatic protons). The proton signal of H-1 was hidden under the hydroxyl peak and appeared as a doublet with $J$=5.8 Hz at $\delta$ 4.83, when recorded at 40 °C. In the $^1$H NMR of the *threo–erythro*-mixture of *15*, obtained by hydrolysis of compound *13a*, the proton signals, arising from the *threo*-isomer, could be seen: $\delta$ 3.82, 3.85 (6 H, 2×s, 2×-OMe), 4.87 (1H, d, $J$=6.0 Hz, H-1).

Acetylation of the *erythro*-isomer of *15* gave the tetraacetate. $^1$H NMR ($CDCl_3$): $\delta$ 1.7–2.1 (2 H, m, $\beta$-$CH_2$-), 2.03, 2.06, 2.10 (9H, 3×s, 3×-OAc), 2.30 (3 H, s, Ar–OAc), 2.63 (2 H, br.t., $J$=7.5 Hz, $\gamma$-$CH_2$-), 3.76, 3.81 (6 H, 2×s, 2×-OMe), 4.07 (2 H, t, $J$=6.5 Hz, $\alpha$-$CH_2$-), 4.0–4.4 (2 H, m, H-3), 4.5–4.7 (1 H, m, H-2), 6.07 (1 H, d, $J$=5.0 Hz, H-1), 6.6–7.1 (6 H, m, aromatic protons). In the $^1$H NMR of the tetraacetate of *threo–erythro-15*, the proton signals of the *threo*-isomer could be seen: $\delta$ 1.7–2.1 (2 H, m, $\beta$-$CH_2$-), 2.0 (3 H, s, -OAc), 2.06 (6 H, s, 2×-OAc), 2.30 (3 H, s, Ar–OAc), 2.63 (2 H, br.t, $J$=7.5 Hz, $\gamma$-$CH_2$-), 3.81, 3.82 (6 H, 2×s, 2×-OMe), 4.07 (2 H, t, $J$=6.5 Hz, $\alpha$-$CH_2$-), 4.0–4.4 (2 H, m, H-3), 4.5–4.7 (1 H, m, H-2), 6.13 (1 H, d, $J$=6.0 Hz, H-1), 6.6–7.1 (6 H, m, aromatic protons).

Methylation of *10* (17 mg) with diazomethane gave a compound (9 mg) which was treated with cellulase at room temperature overnight to yield an aglycone (a *threo–erythro*-mixture), identical with *15*.

Compound *21*, amorphous, $[\alpha]_D^{22}$ −28° (MeOH, c 0.8). $^1$H NMR ($CD_3OD$): $\delta$ 1.8–2.2 (2 H, m, H-8, 8′), 2.5–2.8 (4 H, m, H-7, 7′), 3.1–3.9 (9 H, m), 3.75 (6 H, s, 2×-OMe), 4.14 (1 H, d, $J$=7.0 Hz, xylose 1-H), 6.5–6.8 (6 H, m, aromatic protons).

Acetylation of *21* (7 mg) yielded the hexaacetate (5 mg). $^1$H NMR ($CDCl_3$): $\delta$ 1.8–2.2 (2 H, m, H-8, 8′), 2.03 (3 H, s, -OAc), 2.05 (9 H, s, 3×-OMe), 2.30 (6 H, s, 2×Ar–OAc), 2.5–2.8 (4 H, m, H-7,7′), 3.2–3.6 (2 H, m), 3.77 (6 H, s, 2×-OMe), 3.7–4.4 (4 H, m), 4.41 (1 H, d, $J$=7.0 Hz, xylose 1-H), 4.8–5.4 (3 H, m), 6.5–7.0 (6 H, m, aromatic protons).

Methylation of *21* (5 mg) gave the hexamethyl ether (3 mg). MS (probe 70 eV), *m/z* (rel. int.): 578 ($M^+$, 1), 402 (2), 386 (2), 355 (3), 247 (10), 233 (13), 152 (15), 151 (100), 115 (10), 101 (26), 88 (20), 73 (13), 45 (50).

Compound *23*, amorphous, $[\alpha]_D^{22}$ +9.5° (MeOH, c 0.6) (lit.[12], +10.8°). $^1$H NMR ($CD_3OD$): $\delta$ 2.9–3.2 (2 H, m, H-8, 8′), 3.3–4.0 (8 H, m), 3.84, 3.85 (6 H, 2×s, 2×-OMe), 4.1–4.3 (2 H, m, H-$9_A$, $9_A$′), 4.6–4.8 (2 H, m, H-7, 7′), 6.7–7.2 (6 H, m, aromatic protons). The anomeric proton signal was hidden under the hydroxyl peak and appeared at $\delta$ 4.88 as a doublet, $J$=7.0 Hz when the the spectrum was recorded at 50 °C.

Acetylation of *23* (6 mg) yielded the pentaacetate. $^1$H NMR ($CDCl_3$): $\delta$ 2.04, 2.08 (12 H, 2×s, 4×-OAc), 2.31 (3 H, s, Ar–OAc), 2.9–3.2 (2 H, m, H-8, 8′), 3.5–4.0 (3 H, m), 3.83, 3.85 (6 H, 2×s, 2×-OMe), 4.1–4.5 (4 H, m), 4.7–5.4 (6 H, m), 6.7–7.2 (6 H, m, aromatic protons).

*Acknowledgements.* The authors are grateful to Mr. Rolf Andersson and Mr. Suresh Gohil for recording the NMR and MS spectra. We are also indebted to Mr. Xu Jia-hua (Nanjing Technological College of Forestry, China) for the identification of the *Pinus massoniana* Lamb. needles.

## REFERENCES

1. Shen, Z. and Theander, O. *Phytochemistry* (1984). *In press.*
2. Shen, Z. and Theander, O. *Phytochemistry* (1984). *In press.*
3. Popoff, T. and Theander, O. *Phytochemistry 14* (1975) 2065.
4. Popoff, T. and Theander, O. *Acta Chem. Scand. B 31* (1977) 329.
5. Lundgren, L.N., Popoff, T. and Theander, O. *Phytochemistry 20* (1981) 1967.
6. Miki, K. and Sasaya, T. *Mokuzai Gakkaishi 25* (1979) 437.
7. Imperato, F. *Phytochemistry 22* (1983) 312.
8. Markham, K.R. *Phytochemistry 15* (1976) 147.
9. Higuchi, R. and Donnely, D.M.X. *Phytochemistry 16* (1977) 1587.
10. Takani, M., Ohya, K. and Takahashi, K. *Chem. Pharm. Bull. 27* (1979) 1422.
11. Chiba, M., Hisada, S. and Nishibe, S. *Shoyakugaku Zasshi 32* (*3*) (1978) 194.
12. Kudo, K., Nohara, T., Komori, T., Kawasaki, T. and Schulten, H.-R. *Planta Medica 40* (1980) 250.
13. Lundgren, L.N., Popoff, T. and Theander, O. *Acta Chem. Scand. B 36* (1982) 695.

Received June 25, 1984.