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PHENOLICS FROM INNER BARK OF *PINUS SYLVESTRIS*

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Key Word Index—*Pinus sylvestris*; Pinaceae; inner bark; monoaryl glycoside; stilbene; lignan; flavonoid; catechin; proanthocyanidin.

Abstract—2-*O*-[4′-(α-Hydroxypropyl)-2′-methoxyphenyl]-1-*O*-β-D-xylopyranosyl glycerol and 3′-*O*-methylcatechin, in addition to 24 known phenolic compounds, have been isolated from inner bark of *Pinus sylvestris* and identified on the basis of chemical and spectroscopic evidence.

INTRODUCTION

Norway spruce (*Picea abies*), Scots pine (*Pine sylvestris*), silver birch (*Betula pendula*) and hairy birch (*B. pubescens*) are of great economic importance for the Swedish forest industry and are the predominant tree species in the country. In the last few years, these species have received a considerable amount of attention in our laboratory, with respect to the isolation and characterisation of the low M_r phenolic constituents from their barks. This has resulted in a series of reports [1–5], where a number of monoarylic phenols, diarylheptanoids, stilbenes, lignans, flavonoids, catechins and proanthocyanidins have been the subject of the studies. The present paper is concerned with the inner bark of *P. sylvestris* and reports the identification of some 26 phenolic compounds, two of which have not been reported previously.

RESULTS AND DISCUSSION

The aqueous ethanol extract of fresh inner bark of *P. sylvestris* was fractionated using Sephadex LH-20, silica gel column chromatography and reverse-phase HPLC to afford eight monoaryl compounds (**1–8**), two stilbene glucosides (**9** and **10**), five lignans (**11–15**), four flavonoids (**16–19**), three catechins (**20–22**) and four proanthocyanidins (**23–26**).

Monoaryl and stilbene glycosides

p-Hydroxybenzoic acid β-D-glucopyranoside (**1**), 3,4′-dihydroxy-3′-methoxypropiophenone 3-*O*-β-D-glucopyranoside (**4**), *trans*-coniferyl β-D-glucopyranoside (**5**) and *trans*-*p*-coumaric acid β-D-glucopyranoside (**6**) were identified by direct comparison ($[\alpha]_D$, UV, HPLC and ^{1}H NMR) with authentic samples from inner bark of *B. pubescens* (**1**) or *P. abies* (**4–6**) [2, 5]. Vanillic acid β-D-glucopyranoside (**2**), dihydroconiferin (**3**), *trans*-ferulic acid β-D-glucopyranoside (**7**), 3,4′,5-trihydroxy-*trans*-stilbene 4′-*O*-β-D-glucopyranoside (**9**) and 3,4′-dihydroxy-5-methoxy-*trans*-stilbene 4′-*O*-β-D-glucopyranoside (**10**) were identified by comparison with ^{1}H and ^{13}C NMR data in the literature [6–10]. The stilbenes **9** and **10**, that were isolated in small amounts (25 mg and 5 mg, respectively), have previously been reported from bark of pine [9, 10]. Careful analysis did not reveal any pinosylvin or pinosylvin monomethyl ether in the bark. These stilbenes are well-established in heartwood and are produced in the sapwood of pine in response to fungal infection [11].

Compound **8** ($[\alpha]_D$ −39.1°) was isolated in low amounts (7 mg). Its ^{1}H and ^{13}C NMR spectra revealed three sets of signals, each of which had chemical shifts that compared well with those of dihydroconiferyl alcohol, glycerol and xylose, respectively. A β-xylopyranoside configuration in **8** was based on the ^3J value (*d*, 7.5 Hz) for the anomeric proton signal. A close correspondence between ^{1}H and ^{13}C NMR signals from the dihydroconiferyl alcohol moieties of **8** and **3**, implied that the hydroxyl group of the aromatic ring of the dihydroconiferyl alcohol moiety of **8** was substituted and that of the propyl side-chain was free. Enzymatic (pectinase) hydrolysis of **8** yielded xylose and an aglycone. A D-configuration of xylose was evident as glycoside could be hydrolysed with pectinase. The ^{1}H NMR spectrum of the aglycone revealed signals from a glycerol and dihydroconiferyl alcohol moiety. The spectrum from the glycerol region showed only one set of signals (at δ 3.75) from the methylene protons, indicating that the ether linkage to dihydroconiferyl alcohol was *via* C-2 of glycerol and that xylose was linked to C-1 before the hydrolysis. Acetylation of **8** produced the pentaacetate. On the

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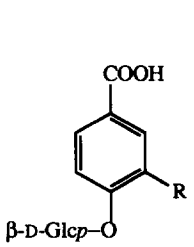
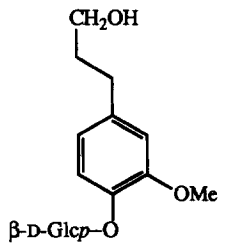


1. R = H
2. R = OMe



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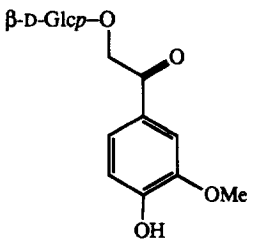

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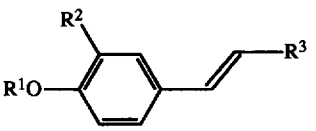
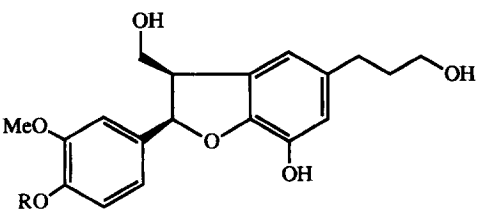


5. R^1 = H; R^2 = OMe; R^3 = CH_2-*O*-β-D-Glc*p*
6. R^1 = β-D-Glc*p*; R^2 = H; R^3 = COOH
7. R^1 = β-D-Glc*p*; R^2 = OMe; R^3 = COOH

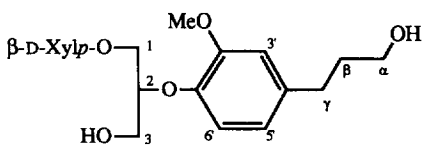
basis of these findings, **8** is identified as 2-*O*-[4′-(α-hydroxypropyl) - 2′ - methoxyphenyl] - 1 - *O* - β - D - xylopyranosyl glycerol. The absolute configuration at C-2 is not known. Compound **8** has not been reported previously.

Lignans, flavonoids, catechins and proanthocyanidins

Lignans **11–13**, **14** (4:5 *threo/erythro* mixture according to the ^{1}H NMR spectrum of the heptaacetate), **15** (1:10 *threo/erythro* mixture according to the ^{1}H NMR spectrum of the pentaacetate of the aglycone [12]), (2*R*,3*R*)-dihydroquercetin (**16**), (2*R*,3*R*)-dihydroquercetin 3′-β-D-glucopyranoside (**17**) and (+)-catechin (**20**) were shown to have data ($[\alpha]_D$, HPLC and ^{1}H NMR) comparable to authentic samples from needles of *P. abies* and *P. sylvestris* [13–15]. It should



11. R^1 = H
12. R = β-D-Glc*p*
13. R = α-L-Rha*p*



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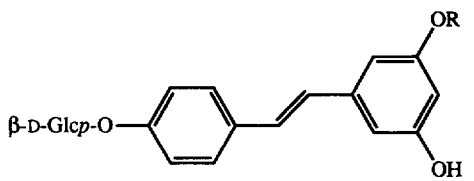
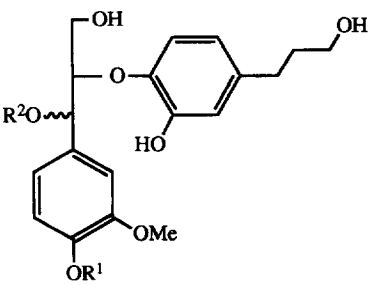
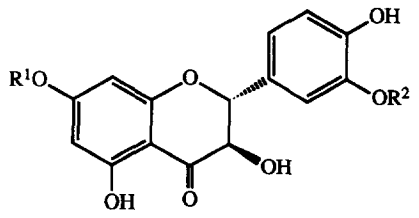


9. R = H
10. R = Me



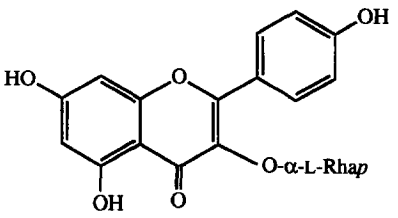
14. R^1 = β-D-Xyl*p*; R^2 = H
15. R^1 = H; R^2 = β-D-Glc*p*



16. $R^1 = R^2 = H$

17. $R^1 = H$; $R^2 = \beta$-D-Glc*p*

18. $R^1 = \beta$-D-Glc*p*; $R^2 = H$



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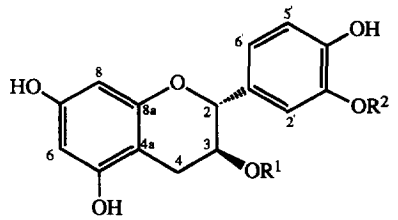


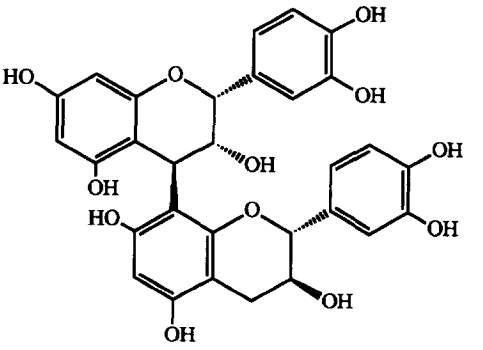
20. $R^1 = R^2 = H$

21. $R^1 = \beta$-D-Glc*p*; $R^2 = H$

22. $R^1 = H$; $R^2 = OMe$

be noted that the *threo*-isomer of **15** was found previously in needles of *P. sylvestris* [16]. Kaempferol 3-β-L-rhamnopyranoside (**19**) and (+)-catechin 3-β-D-glucopyranoside (**21**) were identified by comparison of the $[\alpha]_D$, ^1H and ^{13}C NMR spectral data with literature data [17, 18]. Compounds **23** and **25** were shown to be identical (^1H and ^{13}C NMR spectra of the decaacetates) with proanthocyanidins B1 and B3, respectively, from bark of *P. abies* [5], and compound **24** was identified as proanthocyanidin B2 by comparison of the ^1H NMR spectrum of the decaacetate with literature data [19]. Epicatechin-(4$\beta \rightarrow$8)-epicatechin-(4$\beta \rightarrow$8)-catechin (**26**) was identified ($[\alpha]_D$ and ^{13}C and ^1H NMR of the pentadecaacetates) by comparison with published data [20].

Compound **18** was identified as (2*R*,3*R*)-2,3-dihydroquercetin 7-β-D-glucopyranoside. This was based on a good agreement with literature data [21]



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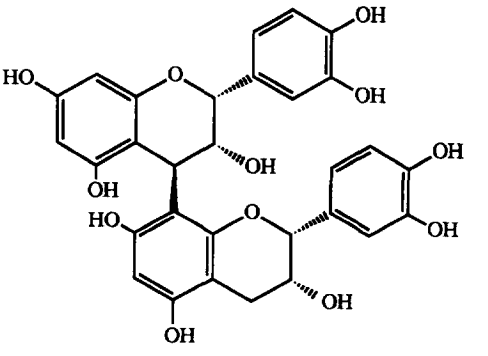

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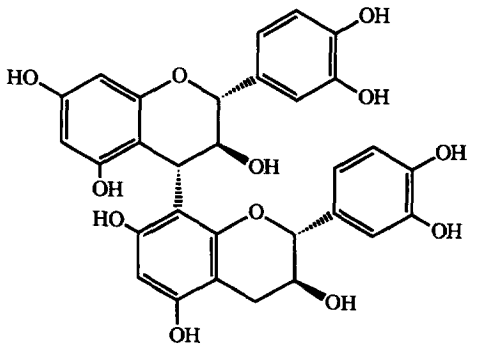
with respect to the optical rotation and the chemical shifts in ^{13}C NMR. However, a comparison of the ^1H NMR data revealed that the aromatic protons of **18** had chemical shifts consistently downfield by 0.2 ppm to those reported [21]. There was a complete agreement of ^1H NMR chemical shifts between the B-rings of **18** and of **16**, however, and this puts the published proton data in doubt.

Compound **22** was identified as 3′-*O*-methylcatechin by comparison (^1H and ^{13}C NMR) with an authentic sample, previously isolated from bark of *P. abies* as the 7-β-D-glucopyranoside [5]. Compound **22** has not been reported previously, and **18**, **19** and **21** have not been found before in *Pinus* species.

EXPERIMENTAL

General. NMR were measured at 400 (^1H) or 101 (^{13}C) MHz. Chemical shifts are given on the δ (ppm) scale with TMS as int. standard. 2D NMR was performed with ^1H-^1H and ^1H-^{13}C COSY, long-range ^1H-^{13}C COSY and NOESY expts. MS were recorded on a quadropole instrument. Optical rotations: 20–23°. For semi-prep. HPLC, a Nova-Pak C_{18} Radial-Pak Cartridge (8 × 100 mm) was used. TLC was performed on silica gel, inspected under UV light and sprayed with 50% H_2SO_4. Enzymatic hydrolysis was carried out in aq. solns with crude pectinase from *Aspergillus niger* (Sigma); aglycones were extracted from EtOAc



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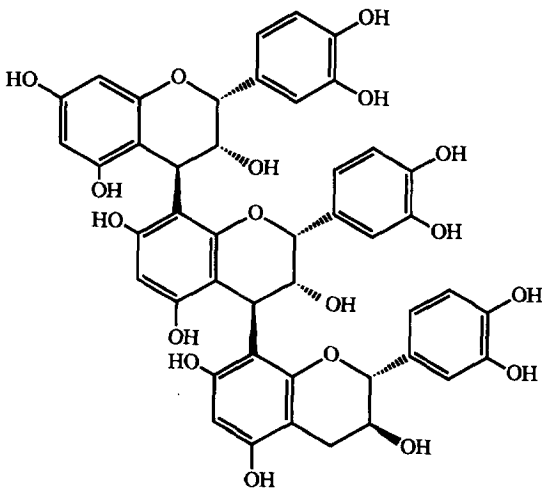

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and sugars in the aq. phase identified as their TMSi derivative by GC.

Isolation. Inner bark (1.0 kg, 0.43 kg dry wt), collected in January near Uppsala, Sweden, from ten-year-old trees, was homogenized with an Ultra Turrax in 95% EtOH (2 × 2500 ml) and 80% EtOH (2 × 2000 ml) at room temp. for 4 × 5 min. Extracts were combined, concd to dryness in *vacuum* at 30° and the residue dild with H_2O (2000 ml) and extracted with petrol (2 × 2000 ml). The aq. phase was concd and chromatographed on Sephadex LH-20 with H_2O, H_2O–EtOH (20–95% stepwise increasing EtOH content) and aq. 70% Me_2CO as eluents. Eleven frs (A–K) were obtained. Sephadex G-15 (H_2O), silica gel CC (EtOAc–MeCOEt–MeOH–H_2O, 6:2:1:1) and semi-preparative HPLC (MeOH–H_2O–HOAc, 15:84:1) yielded from fr. A, **1** (12 mg) and **2** (10 mg). Silica gel CC of frs B and C ($CHCl_3$–MeOH–H_2O and EtOAc–MeCOEt–MeOH–H_2O in different proportions) yielded from fr. B, **3** (361 mg), **4** (130 mg), **5** (25 mg), **6** (25 mg), **7** (55 mg) and **8** (7 mg), and from fr. C, **11** (40 mg), **14** (25 mg) and **15** (78 mg). Silica gel CC of frs D–I ($CHCl_3$–MeOH–H_2O, $CHCl_3$–MeCOEt–MeOH–HOAc and EtOAc–MeCOEt–MeOH–H_2O in different proportions) yielded from frs D–F, **12** (43 mg), **13** (7 mg), **17** (300 mg), **18** (15 mg), **20** (1650 mg) and **21** (20 mg), and from G–I, **9** (25 mg), **10** (5 mg), **16** (17 mg), **19** (6 mg) and **22** (14 mg). Sephadex LH-20 CC of frs J and K (H_2O, 20–95% stepwise increasing EtOH content and 70% Me_2CO) yielded **23** (1100 mg), **26** (25 mg) and a mixt. of **24** and **25**. The mixt. was acetylated (Ac_2O–pyridine) and refractionated on silica gel ($CHCl_3$–MeOH 50:1) yielding the decaacetates of **24** (32 mg) and **25** (28 mg).

Compound **8**. $[\alpha]_D$ −39.1° (MeOH, *c* 0.7). UV λ_{max} nm: 224, 277 nm; ^{1}H NMR (CD_3OD): δ 1.82 (2H, *m*, H-β), 2.63 (2H, *t*, H-γ), 3.18 (1H, *dd*, H-5″b), 3.19 (1H, *dd*, H-2″), 3.31 (H-3″; signal overlapped with solvent), 3.48 (1H, *ddd*, H-4″), 3.56 (2H, *t*, H-α), 3.78 (1H, *dd*, H-1b), 3.81 (2H, *m*, H-3), 3.84 (1H, *dd*, H-5″a), 3.84 (3H, *s*, OMe), 3.99 (1H, *dd*, H-1a), 4.26 (1H, *dd*, H-1″), 4.31 (1H, *m*, H-2), 6.73 (1H, *dd*, H-5′), 6.85 (1H, *d*, H-3′), 6.99 (1H, *d*, H-6′); *J*: 3′,5′ = 2.0; 5′,6′ = 8.1; α,β = 6.5; β,γ = 7.8; 1a,b = 10.7; 1a,2 = 4.4; 1b,2 = 2.0; 1″,2″ = 7.5; 2″,3″ = 9.0; 3″,4″ = 8.7; 4″,5″a = 5.3; 4″,5″b = 10.2; 5″a,5″b = 11.5 Hz. ^{13}C NMR (CD_3OD): δ 32.7 (C-γ), 35.6 (C-β), 56.4 (OMe), 62.2 (C-α and C-3), 66.9 (C-5″), 68.8 (C-1), 71.2 (C-4″), 74.9 (C-2″), 77.7 (C-3″), 81.3 (C-2), 105.4 (C-1″), 114.0 (C-3′), 119.4 (C-6′), 121.8 (C-5′), 138.4 (C-4′), 146.5 (C-1′), 151.9 (C-2′). EI-MS *m/z* (rel. int.): 388 $[M]^+$ (6), 182 (100), 164 (12), 138 (40), 137 (73), 103 (20), 97 (19), 73 (39). Aglycone of **8**. ^{1}H NMR (CD_3OD): δ 1.82 (2H, *m*, H-β), 2.64 (2H, *t*, H-γ), 3.57 (2H, *t*, H-α), 3.75 (4H, *m*, H-1 and H-3), 3.85 (3H, *s*, OMe), 4.16 (1H, *m*, H-2), 6.74 (1H, *dd*, H-5′), 6.86 (1H, *d*, H-3′), 6.99 (1H, *d*, H-6′); *J*: 3′,5′ = 2.0; 5′,6′ = 8.2; α,β = 6.4; β,γ = 7.8 Hz. Acetylation (Ac_2O–pyridine) of **8** yielded the pentaacetate. ^{1}H NMR ($CDCl_3$): δ 1.94 (2H, *m*, H-β), 2.03, 2.05, 3 × 2.06 (5*s*, 5OAc), 2.63 (2H, *t*, H-γ), 3.37 (1H, *dd*, H-5″b), 3.75 (1H, *dd*, H-1b), 3.83 (3H, *s*, OMe), 4.03 (1H, *dd*, H-1a), 4.08 (2H, *t*, H-α), 4.12 (1H, *dd*, H-5″a), 4.31 (2H, *m*, H-3), 4.44 (1H, *m*, H-2), 4.60 (1H, *d*, H-1″), 4.93 (2H, *m*, H-2″ and 4″), 5.16 (1H, *t*, H-3″), 6.68 (1H, *dd*, H-5′), 6.71 (1H, *d*, H-3′), 6.90 (1H, *d*, H-6′); *J*: 3′,5′ = 1.8; 5′,6′ = 8.0; α,β = 6.4; β,γ = 7.7; 1a,b = 10.7; 1a,2 = 4.2; 1b,2 = 5.6; 1″,2″ = 6.7; 2″,3″ = 8.3; 3″,4″ = 8.3; 4″,5″a = 4.9; 4″,5″b = 8.5; 5″a,5″b = 11.9 Hz. EI-MS *m/z* (rel. int.): 598 $[M]^+$ (3), 375 (13), 259 (5), 157 (23), 145 (42), 43 (100).

Compound **18**. ^{1}H NMR (CD_3OD): δ 3.35–3.46 (4H, *m*, H-2″-H-5″), 3.67 (1H, *dd*, H-6″b), 3.86 (1H, *dd*, H-6″a), 4.55 (1H, *d*, H-3), 4.94 (1H, *d*, H-2), 4.95 (1H, *d*, H-1″), 6.19 (1H, *d*, H-6), 6.21 (1H, *d*, H-8), 6.79 (1H, *d*, H-5′), 6.84 (1H, *dd*, H-6′), 6.96 (1H, *d*, H-2′); *J*: 2,3 = 11.7; 6,8 = 2.2; 2′,6′ = 1.9; 5′,6′ = 8.2; 1″,2″ = 7.2; 5″,6″a = 2.1; 5″,6″b = 5.5; 6″a,6″b = 12.1 Hz. ^{13}C NMR (CD_3OD): δ 62.3 (C-6″), 71.1 (C-4″), 73.8 (C-3), 74.6 (C-2″), 77.7(C-5″), 78.2 (C-3″), 85.3 (C-2), 97.0 (C-6), 98.2 (C-8), 101.2 (C-1″), 103.4 (C-4a), 115.9 (C-2′), 116.0 (C-5′), 120.9 (C-6′), 129.6

(C-1′), 146.3 (C-3′), 147.1 (C-4′), 164.2 (C-5), 164.7 (C-8a), 167.2 (C-7), 199.2 (C-4). Acetylation (Ac_2O–pyridine) of **18** yielded the octaacetate. ^1H NMR ($CDCl_3$): δ 1.98, 2.04, 2 × 2.05, 2.06 (5*s*, 5OAc), 2 × 2.31, 2.37 (3*s*, 3OAc), 3.90 (1H, *m*, H-5″), 4.18–4.23 (2H, *m*, H-6″a, H-6″b), 5.13–5.29 (4H, *m*, *H*-1″ − *H*-4″), 5.38 (1H, *d*, H-3), 5.65 (2H, *d*, H-2), 6.40 and 6.54 (2H, 2 × *d*, H-6 and H-8), 7.26 (1H, *d*, H-5′), 7.28 (1H, *d*, H-2′), 7.36 (1H, *dd*, H-6′); *J*: 2,3 = 12.2; 6,8 = 2.4; 2′,6′ = 2.0; 5′,6′ = 8.3 Hz.

Compound **22**. $[\alpha]_D$ +21.4° (50% aq. Me_2CO, *c* 0.3). UV λ_{max} nm: 280 nm. ^1H NMR (CD_3OD): δ 2.51 (1H, *dd*, H-4b), 2.89 (1H, *dd*, H-4a), 3.84 (3H, *s*, 3′-OMe), 4.00 (1H, *ddd*, H-3), 4.58 (1H, *d*, H-2), 5.85 (1H, *d*, H-6), 5.93 (1H, *d*, H-8), 6.79 (1H, *d*, H-5′), 6.83 (1H, *dd*, H-6′), 6.97 (1H, *d*, H-2′); *J*: 2,3 = 7.8, 3,4a = 5.5, 3,4b = 8.5; 4a,4b = 16.2; 6,8 = 2.3; 2′,6′ = 2.0, 5′,6′ = 8.1 Hz. ^{13}C NMR (CD_3OD): δ 29.0 (C-4), 56.4 (3′-OMe), 68.9 (C-3), 83.1 (C-2), 95.5 (C-6), 96.3 (C-8), 100.9 (C-4a), 111.9 (C-2′), 116.0 (C-5′), 121.3 (C-6′), 132.0 (C-1′), 147.5 (C-4′), 148.8 (C-3′), 156.9 (C-5), 157.5 (C-8a), 157.8 (C-7).

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