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PHENOLIC EXTRACTIVES FROM ROOT BARK OF *PICEA ABIES*

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Key Word Index—*Picea abies*; Pinaceae; root bark; monoaryl glycosides; stilbenes; lignans; flavonoids; proanthocyanidins.

Abstract—Five new compounds, viz. 3,4-dimethoxyphenyl 2-*O*-(3-*O*-methyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside, 4′-hydroxyphenacyl β-D-glucopyranoside, (2*R*,3*R*)-2,3-dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol 4′-*O*-(3-*O*-methyl-α-L-rhamnopyranoside), 1-(4′-hydroxy-3′-methoxyphenyl)-2-[4″-(3-hydroxypropyl)-2″-methoxyphenoxy]-1,3-propanediol 4′-*O*-β-D-xylopyranoside and 3′-*O*-methylcatechin 7-*O*-β-D-glucopyranoside, together with several known compounds were isolated from the root bark of *Picea abies*. The structures were elucidated on the basis of chemical and spectroscopic evidence.

INTRODUCTION

The root rot fungus '*Heterobasidion annosum*' is a serious pathogen on Norway spruce (*Picea abies* (L.) Karst.) in Sweden. The hyphal growth is preferentially concentrated in the central part of the root and stem while the living wood (sapwood) and bark of the tree have a considerable resistance to the fungus. Little is known about the reasons why sapwood and bark can resist hyphal attack, but the presence of extractives that are toxic to the fungus is probably of vital importance. Spruce bark is known to have a high content of secondary metabolites, especially of stilbene glucosides and various resin acids. Recent studies [1] at this University on the importance of bark extractives for hyphal penetration of *H. annosum* showed that a high level of the stilbene 'astringin' favoured resistance to the fungus, while there was no correlation between the initial concentration of resin acids in the bark and the depth of hyphal penetration. Within the scope of this project, we have made a broad screening of phenolic and terpenoid constituents in the root bark of *P. abies*. A wider knowledge in this field was considered to be a prerequisite for further investigations in this laboratory concerning spruce bark extractives and their effect on *H. annosum*. The present paper deals with the isolation and identification of various types of phenolic compounds. A presentation on the terpenoid constituents of spruce bark will be published elsewhere.

RESULTS AND DISCUSSION

An ethanol extract of the fresh root bark from Norway spruce was subjected to Sephadex LH-20 and silica gel column chromatography and reverse-phase HPLC to afford nine monoaryl compounds (**1**–**9**), three stilbene glucosides (**10**–**12**), 10 lignans (**13**–**22**), two flavonoids (**23**, **24**), and five catechins and proanthocyanidins (**25**–**29**).

Monoaryl and stilbene compounds

The already known tachioside (**1**), 3,4′-dihydroxy-3′-methoxypropiophenone 3-*O*-β-D-glucopyranoside (**6**), *trans*-coniferyl β-D-glucopyranoside (**7**), *trans*-*p*-coumaric acid (**8**), in addition to the stilbene glucosides, piceid (**10**), astringin (**11**) and isorhapontin (**12**), were identified by direct comparison ($[\alpha]_D$, TLC, ^{1}H and ^{13}C NMR) with authentic samples [2–4]; 3,4′-dihydroxypropiophenone 3-*O*-β-D-glucopyranoside (**5**) and *trans*-*p*-coumaric acid 4-*O*-glucoside (**9**) were identified by comparison with literature data [5, 6]. However, the ^{13}C NMR chemical shifts for **5** were consistently observed *ca* 2 ppm downfield from those reported [5].

Compound **2** was identified as 3,4-dimethoxyphenyl β-D-glucopyranoside. The ^{13}C NMR spectrum corresponded closely to literature data [7] and enzymatic hydrolysis yielded 3,4-dimethoxyphenol and glucose.

The ^{1}H and ^{13}C NMR spectra of **3** revealed signals similar to those from **2** and also signals from a rhamnose (presumably L-rhamnose) residue and from an aliphatically linked methoxyl group. Trifluoracetic acid hydrolysis yielded 3,4-dimethoxyphenol, glucose and 3-*O*-methyl rhamnose. Identification of monosaccharides was based on GC-mass spectrometry of the alditol acetate derivatives [8]. A downfield shift of the rhamnose C-3 signal (+ 9.5 ppm) and upfield shifts of the C-2 and C-4 signals (− 3.9 and 0.9 ppm, respectively) in the ^{13}C NMR spectrum of **3**, in comparison with α-L-rhamnopyranose [9],

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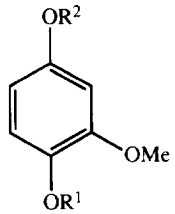
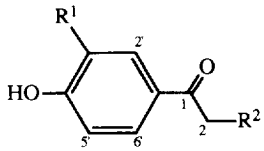
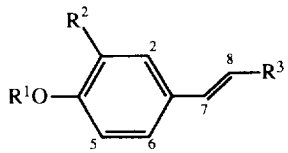


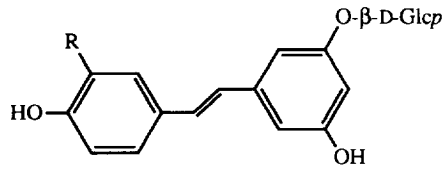
1. R^1 = H; R^2 = β-D-Glc*p*
2. R^1 = Me; R^2 = β-D-Glc*p*
3. R^1 = Me; R^2 = 3-*O*-Me-α-L-Rha*p*-(1→2)-β-D-Glc*p*



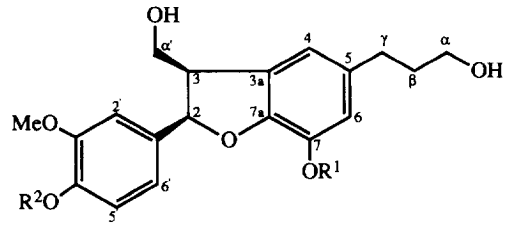
4. R^1 = H; R^2 = *O*-β-D-Glc*p*
5. R^1 = H; R^2 = CH_2-*O*-β-D-Glc*p*
6. R^1 = OMe; R^2 = CH_2-*O*-β-D-Glc*p*



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



10. R = H
11. R = OH
12. R = OMe



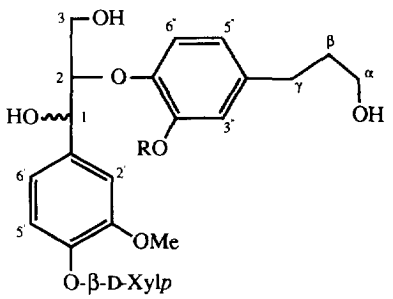
13. R^1 = R^2 = H
14. R^1 = H; R^2 = β-D-Glc*p*
15. R^1 = H; R^2 = α-L-Rha*p*
16. R^1 = OMe; R^2 = β-D-Glc*p*
17. R^1 = OMe; R^2 = α-L-Rha*p*
18. R^1 = H; R^2 = 3-*O*-Me-α-L-Rha*p*

and a cross-peak in the NOESY analysis between the methoxyl group and rhamnose H-3, further confirmed the presence of a methoxyl group at rhamnose C-3. In the ^{13}C NMR spectrum, the C-2 and C-3 signals from the glucose moiety appeared downfield (+ 3.9 and + 1.1 ppm, respectively) and the C-1 signal upfield (− 1.8 ppm) in comparison with **2**; the NOESY diagram showed cross-peaks between signals for the H-2 of glucose and H-1 of the rhamnose moiety. Hence, rhamnose is apparently attached at C-2 of the glucose moiety. A β-D-glucopyranosyl configuration in **3** was based on the ^3J values (7.6 Hz) for the glucose anomeric proton signal, and the ^{1}H and ^{13}C NMR chemical shift signals of H-5 and C-5 (δ_H 4.12, δ_C 69.9) of the rhamnose moiety suggested an α-L-rhamnopyranosyl configuration [9]. Therefore, **3** is 3,4-dimethoxyphenyl 2-*O*-(3-*O*-methyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside, a novel compound.

Compound **4** (CI-mass spectrum *m/z* 332 [M + NH_4]$^+$) was isolated in small amounts (8 mg) by reversed-phase HPLC. The ^{13}C NMR spectrum revealed signals from a carbonyl group, a *para*-disubstituted aromatic ring, a glucose moiety and a methylene carbon at δ72.1. The ^{1}H NMR spectrum showed signals from an anomeric proton at δ4.38 (J = 7.5 Hz), consistent with an alkyl β-glucopyranoside, and from a methylene group (δ4.90 and 5.26; *d*, J = 16.9 Hz). According to the observed chemical shifts and the large geminal-coupling between the methylene protons, in addition to the presence of a cross-peak in the long-range ^{1}H-^{1}H COSY diagram between the anomeric proton and the methylene group, a linkage between the latter and both the carbonyl and the anomeric carbon was suggested. A cross-peak in the long-range ^{1}H–^{1}H COSY diagram between the signals for the anomeric proton and those of the methylene protons supported this suggestion. Because the regions for the aromatic protons in the ^{1}H NMR spectra of **4** and authentic 4-hydroxyacetophenone were almost identical, **4** is 4′-hydroxyphenacyl β-D-glucopyranoside.

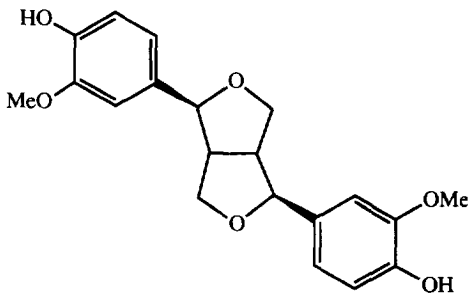
Lignans

The lignans **13**–**17**, **19** (3:1 *erythro/threo* mixture according to the ^{1}H NMR spectrum of the heptaacetate [10]) and (+)-pinoresinol (**21**) were identified by direct comparison ($[\alpha]_D$, TLC and ^{1}H NMR) with authentic



19. R = H

20. R = Me



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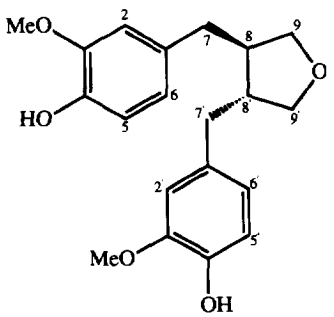

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samples from needles of *Picea abies* or *Pinus massoniana* [10, 11].

Compound **18** yielded the aglycone **13** and 3-*O*-methyl rhamnose on trifluoroacetic acid hydrolysis; acetylation yielded the pentaacetate. The aglycone parts of the ^1H and ^{13}C NMR spectra of **18** and **15** were virtually identical, and the ^{13}C NMR spectra of the sugar moiety of **18** and the 3-*O*-methyl-α-L-rhamnopyranosyl moiety of **3** were in excellent accordance. On the basis of these findings, we propose the structure (2*R*, 3*R*)-2,3-dihydro-7-hydroxy-2-(4′-hydroxy-3′-methoxyphenyl)-3-hydroxymethyl-5-benzofuranpropanol 4′-*O*-(3-*O*-methyl-α-L-rhamnopyranoside) for **18**.

Compound **20** was isolated as a mixture of its *threo*- and *erythro*-isomers in the ratio of 1:7. The ^1H and ^{13}C NMR spectra of **19** and **20** were similar but revealed for the latter compound a signal from an additional methoxyl group (δ_H 3.77, δ_c 56.5). Cross-peaks in the long-range ^1H–^1H COSY analysis of **20** from the H-3″ signal at δ6.77 on one hand, to H-γ signal at δ2.59 and the methoxyl group signal at δ3.77, suggested a linkage of the latter group to C-2″. The ^1H NMR spectrum of the hexaacetate of **20** showed six aliphatic acetyl groups. Thus, **20** was identified as 1-(4′-hydroxy-3′-methoxyphenyl)-2-[4″-(3-hydroxypropyl)-2″-methoxyphenoxy]-1,3-propandiol 4′-*O*-β-D-xylopyranoside.

Compound **22** was identified as (−)-*trans*-3,4-divanillyltetrahydrofuran; the ^1H NMR and mass spectral data corresponded closely to those for the racemic compound [12]. The mp (105–108°) and $[\alpha]_D$ −43° were quite similar to those of (−)-*trans*-3,4-divanillyltetrahydrofuran (mp 116-117°; $[\alpha]_D$ −52°) [13], formed by hydrogenation of (−)-liovil.

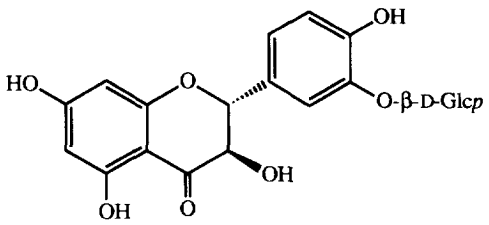
To our knowledge, **18** and **20** have not been found before, and **22** has not been reported previously in a pure state as a natural product.

Flavonoids and proanthocyanidins

Dihydroquercetin 3′-*O*-β-D-glucopyranoside (**23**) and catechin (**25**) were identified by direct comparison ($[\alpha]_D$, TLC and ^1H NMR) with authentic samples from needles of *Pinus sylvestris* [14], and isorhamnetin 3-*O*-(6″-*O*-acetyl)-β-D-glucopyranoside (**24**) by comparison ($[\alpha]_D$ and ^1H NMR) with literature data [15, 16].

Compound **26** was obtained in small amounts (10 mg). Enzymatic hydrolysis yielded an aglycone and D-glucose; acetylation yielded the heptaacetate. The ^1H and ^{13}C NMR spectra of **26** exhibited signals characteristic of a glucose moiety, a flavan-3-ol unit and a methoxyl group (δ_H 3.84, δ_C 56.4). The ^{13}C NMR resonances of the flavan-3-ol A-ring, as well as of the glucose moiety corresponded closely to data for catechin 7-*O*-β-D-glucopyranoside [17]. The long-range ^1H-^1H COSY diagram revealed cross-peaks between the signals for the H-8 and the anomeric proton, and between the methoxyl group and H-2′, confirming the attachment of the glucose moiety to the hydroxyl group at C-7, and suggesting a linkage of the methoxyl group to the C-3′ position of the B-ring. Thus, **26** is 3′-*O*-methylcatechin 7-*O*-β-D-glucopyranoside. Compound **26** has not been reported previously, but the related 3′-*O*-methylepicatechin 7-*O*-β-D-glucopyranoside has been found in bark of Douglas fir [17] and in *Symplocos uniflora* [18].

Compounds **27** and **28** were eluted from the Sephadex LH-20 column with 70% aqueous acetone and identified as the proanthocyanidins B1 and B3, respectively, by comparison of the ^1H NMR spectra of their decaacetates with literature data [19]. During fractionation of the bark extract on Sephadex LH-20, a phenolic fraction emerged from the column in the first effluent (H_2O as mobile phase). Rechromatography of this fraction on the same column material (stepwise elution with H_2O, 96% EtOH and 70% aq. Me_2CO), followed by evaporation and freeze-drying of the 70% aq. Me_2CO fraction, yiel-



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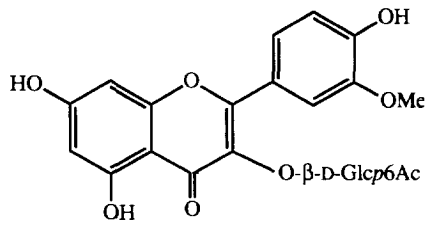

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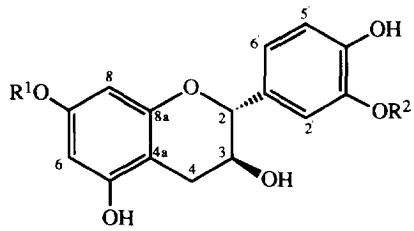
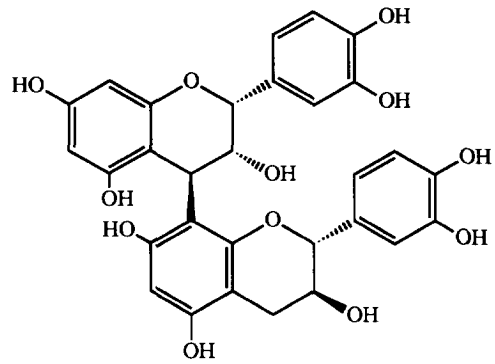


25. $R^1 = R^2 = H$

26. R^1 = β-D-Glc*p*; R^2 = Me



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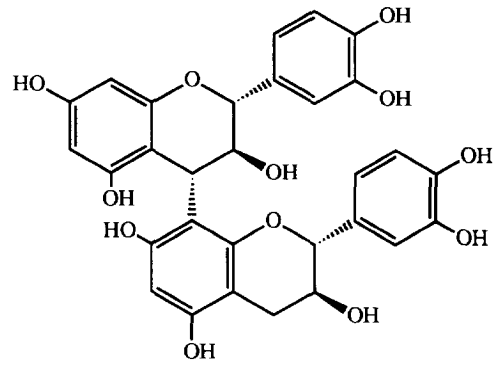

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ded a flavan-3-ol proanthocyanidin polymer (**29**); its ^{13}CNMR spectrum was in accordance with literature spectra [19, 20]. The average $M_r \approx 3000$ was calculated from the intensity ratio of the C-3 signals from the internal and terminal units. About 70% of the monomer units of the polymer possessed 2,3-*cis*-stereochemistry, as determined from the intensity ratio of the C-2 signals from 2,3-*cis* and 2,3-*trans* units [20, 21]. It should be noted that Sephadex LH-20 is known to have a strong affinity for proanthocyanidins under aqueous conditions, and that the unexpected elution of **29** is probably due to overloading of the column.

EXPERIMENTAL

NMR spectra were measured at 400 (^1H) or 101 (^{13}C) MHz. Chemical shifts are given on the δ scale with TMS as int. standard. 2D-NMR was performed by ^1H-^1H, ^1H-^{13}CCOSY and NOESY. MS: quadropole instrument. CI-MS: NH_3 as reaction gas. UV spectra: MeOH. Optical rotations: 20–23°. For semi-prep. HPLC, a Nova-Pak C_{18} Radial-Pak Cartridge (8 × 100 mm) was used. Mp: uncorr. TLC was performed on silica gel plates, inspected under UV light and sprayed with 50% H_2SO_4. Enzymatic hydrolysis was performed in aq. soln with crude pectinase from *Aspergillus niger* (Sigma). The aglycone was extracted with EtOAc and the sugar in the aq. phase was identified as its TMSi derivative by GC. Compounds **3** and **18** were hydrolysed in 2.0 M TFA at 120° for 2 hr and sugars in the reaction mixt. were identified as their alditol acetates by GC-MS [8]. Absolute configurations of the monosaccharides D-glucose and D-xylose of the glycosides have been proposed, because the glycosides were hydrolysed with pectinase.

Isolation. Root bark (5–10 cm root diameter; 2.4 kg, 1.08 kg dry wt), collected in December near Uppsala, Sweden, was homogenized with an Ultra Turrax in 96% EtOH (2 × 6 l) and 80% EtOH (2 × 6 l) at room temp. for 4 × 5 min. The extracts were combined and concd to dryness *in vacuo* at 30°. The residue (359 g) was chromatographed on Sephadex LH-20 with H_2O, H_2O–EtOH (20–96% stepwise increasing EtOH content) and aq. 70% Me_2CO as eluents. Eleven frs (A–K) were obtained. Sephadex LH-20 CC of fr. A (H_2O, 96% EtOH and 70% aq. Me_2CO as eluents), followed by evapn and freeze-drying of the 70% aq. Me_2CO eluate, yielded **29** as a light tan fluffy solid [1.25 g; $[\alpha]_D$ −29° (H_2O; *c* 0.9)]. Silica gel CC yielded from fr. C (EtOAc–MeCOEt–MeOH–HOAc, 9:2:2:1 and EtOAc–MeOH–H_2O–HOAc, 60:9:3:2) **1** (11 mg), **2** (25 mg), **3** (15 mg), **4** (8 mg), **5** (31 mg), **7** (25 mg), **19** (45 mg) and **20** (12 mg); from fr.

D ($CHCl_3$–MeOH–H_2O, 30:10:1, and EtOAc–MeOH–HOAc–H_2O, 60:9:2:3), **14** (110 mg), **15** (79 mg) and **16** (22 mg); from fr. E ($CHCl_3$–MeOH–H_2O, 60:15:1, and EtOAc–MeOH–HOAc–H_2O, 40:5:1:1), **6** (4 mg), **9** (4 mg), **17** (5 mg) and **18** (25 mg); from fr. F ($CHCl_3$–MeOH–H_2O, 60:15:1, and EtOAc–MeCOEt–MeOH–H_2O, 60:20:8:5), **23** (356 mg) and **26** (10 mg); from fr. G ($CHCl_3$–MeOH–H_2O, in different proportions), **8** (10 mg), **13** (14 mg), **21** (36 mg), **22** (20 mg), **24** (8 mg) and **25** (465 mg). Compounds **10** (2.5 g), **11** (5.8 g) and **12** (8.9 g) were isolated from frs G and H ($CHCl_3$–MeOH–H_2O, in different proportion). Compounds **1–5** were further purified by semi-prep. HPLC with aq. MeOH as mobile phase. Fr. K was refractionated on Sephadex LH-20 (H_2O, 96% EtOH and 70% aq. Me_2CO). Evapn and silica gel CC of the 70% aq. Me_2CO eluate (EtOAc–MeOH–H_2O–HOAc, 60:9:3:2), yielded **27** (1.2 g) and **28** (0.7 g).

Compound **2**. Mp 161–163°. $[\alpha]_D$ − 54° (MeOH; *c* 0.4). UV λ_{max} nm: 227, 282. ^{1}H NMR (CD_3OD): δ3.40–3.55 (4H, *m*, H-2′–H-5′), 3.77 (1H, *dd*, H-6′b), 3.86 (3H, *s*, OMe-4), 3.89 (3H, *s*, OMe-3), 3.98 (1H, *dd*, H-6′a), 4.86 (1H, *d*, H-1′), 6.75 (1H, *dd*, H-6), 6.90 (1H, *d*, H-2), 6.93 (1H, *d*, H-5); *J*: 2,6 = 2.7, 5,6 = 8.7, 1′,2′ = 7.6, 5′,6′a = 2.2, 5′,6′b = 5.8, 6′a,6′b = 11.9 Hz. ^{13}C NMR (CD_3OD): δ56.5 (OMe-3), 57.2 (OMe-4), 62.6 (C-6′), 71.5 (C-4′), 75.0 (C-2′), 78.1 (C-3′), 78.2 (C-5′), 103.4 (C-1′), 104.1 (C-2), 109.3 (C-5), 114.0 (C-6), 146.0 (C-1), 151.1 (C-4), 153.9 (C-3). Aglycone of **2**. ^{1}H NMR (CD_3OD): δ3.74, 3.78 (2*s*, 2OMe), 6.30 (1H, *d*, H-6), 6.45 (1H, *d*, H-2), 6.77 (1H, *dd*, H-5); *J*: 2,6 = 2.7, 5,6 = 8.5 Hz.

Compound **3**. $[\alpha]_D$ − 63° (MeOH; *c* 0.7). UV λ_{max} nm: 225 sh, 283. ^{1}H NMR (CD_3OD): δ1.30 (3H, *d*, H-6″), 3.30 (1H, *dd*, H-3″), 3.32 (1H, *m*, H-4′), 3.40 (3H, *s*, OMe-3″), 3.41 (1H, *m*, H-5′), 3.47 (1H, *t*, H-4″), 3.57 (1H, *dd*, H-3′), 3.63 (1H, *dd*, H-2′), 3.67 (1H, *dd*, H-6′b), 3.77 (3H, *s*, OMe-4), 3.81 (3H, *s*, OMe-3), 3.90 (1H, *dd*, H-6′a), 4.12 (1H, *dq*, H-5″), 4.15 (1H, *dd*, H-2″), 4.88 (1H, *d*, H-1′), 5.31 (1H, *d*, H-1″), 6.64 (1H, *dd*, H-6), 6.75 (1H, *d*, H-2), 6.85 (1H, *d*, H-5); *J*: 2,6 = 2.8, 5,6 = 8.9, 1′,2′ = 7.6, 2′,3′ = 8.4, 3′,4′ = 9.3, 5′,6′a = 2.3, 5′,6′b = 5.5, 6′a,6′b = 12.0, 1″,2″ = 1.8, 2″,3″ = 3.2, 3″,4″ = 9.6, 4″,5″ = 9.6, 5″,6″ = 6.2 Hz. ^{13}C NMR (CD_3OD): δ18.2 (C-6″), 56.5 (OMe-3), 57.2 (OMe-4 and OMe-3″), 62.6 (C-6′), 68.0 (C-2″), 69.9 (C-5″), 71.7 (C-4′), 72.7 (C-4″), 78.1 (C-5′), 78.9 (C-2′), 79.2 (C-3′), 82.0 (C-3″), 101.6 (C-1′), 102.2 (C-1″), 103.6 (C-2), 108.5 (C-5), 114.2 (C-6), 145.9 (C-1), 151.2 (C-4), 153.8 (C-3). CI-MS *m/z* (rel. int.): 494 $[M + NH_4]^+$ (22), 340 (25), 315 (20), 194 (38), 178 (100). Acetylation (Ac_2O–pyridine) of **3** yielded the pentaacetate. ^{1}H NMR ($CDCl_3$): δ1.21 (3H, *d*, H-6″), 2.02, 2.06, 2.08, 2 × 2.13 (5*s*, 5OAc), 3.31 (3H, *s*, OMe-3″), 3.53 (1H, *dd*, H-3″), 2 × 3.86 (2*s*, 2OMe), 3.95 (1H, *dd*, H-2′), 4.10–5.33 (9H), 4.57 (1H, *d*, H-1′), 6.58 (1H, *dd*, H-6), 6.62 (1H, *d*, H-2), 6.80 (1H, *d*, H-5); *J*: 2,6 = 2.8, 5,6 = 8.9, 1′,2′ = 7.9, 2′,3′ = 9.6, 2″,3″ = 3.5, 3″, 4″ = 10.1, 5″,6″ = 6.0 Hz.

Compound **4**. $[\alpha]_D$ − 33° (MeOH; *c* 0.2). UV λ_{max} nm: 281. ^{1}H NMR (CD_3OD): δ3.2–3.4 (4H, *m*, H-2″–H-5″), 3.65 (1H, *dd*, H-6″b), 3.89 (1H, *dd*, H-6″a), 4.38 (1H, *d*, H-1″), 4.90 (1H, *d*, H-2b), 5.26 (1H, *d*, H-2a), 6.84 (2H, *d*, H-3′, H-5′), 7.88 (2H, *d*, H-2′, H-6′); *J*: 2a,2b = 16.9, 2′,3′ = 5′,6′ = 8.8, 1″,2″ = 7.5, 5″,6″a = 2.0, 5″,6″b = 5.8, 6″a,6″b = 11.8 Hz. ^{13}C NMR (CD_3OD): δ62.9 (C-6″), 71.7 (C-4″), 72.1 (C-2), 75.1 (C-2″), 77.8 (C-5″), 78.3 (C-3″), 104.4 (C-1″), 116.7 (C-3′,5′), 128.5 (C-1′), 131.7 (C-2′,6′), 164.8 (C-4′), 196.6 (C-1). CI-MS *m/z* (rel. int.): 332 $[M + NH_4]^+$ (37), 315 $[M + H]^+$ (4), 238 (48), 198 (66), 180 (55), 170 (98), 154 (100).

Compound **18**. $[\alpha]_D$ − 25° (MeOH; *c* 0.4). UV λ_{max} nm: 281. ^{1}H NMR (CD_3OD): δ1.21 (3H, *d*, H-6″), 1.78 (2H, *m*, H-β), 2.56 (2H, *t*, H-γ), 3.45 (1H, *m*, H-3), 3.49 (1H, *t*, H-4″), 3.50 (3H, *s*, OMe-3″), 3.54 (1H, *dd*, H-3″), 3.55 (2H, *t*, H-α), 3.81 (3H, *s*, OMe-3′), 3.74 (1H, *dd*, H-α′b), 3.82 (1H, *m*, H-5″), 3.85 (1H, *dd*, H-α′a), 4.26 (1H, *dd*, H-2″), 5.36 (1H, *d*, H-1″), 5.55 (1H, *d*, H-2), 6.57 (1H, *d*, H-4), 6.59 (1H, *d*, H-6), 6.94 (1H, *dd*, H-6′), 7.06 (1H, *d*, H-2′), 7.08 (1H, *d*, H-5′); *J*: 2′,6′ = 1.8, 5′,6′ = 8.2, 2,3 = 5.8, 3,α′a = 5.2, 3,α′b = 7.6, α′a,α′b = 11.0, 4,6 = 1.5, α,β = 6.6, β,γ = 6.4, 1″,2″ = 1.8, 2″,3″ = 3.3, 3″,4″ = 10.0, 4″,5″ = 10.0, 5″,6″ = 6.1 Hz. ^{13}C NMR (CD_3OD): δ18.0 (C-6″), 32.7 (C-γ), 35.8 (C-β), 56.0 (C-3), 56.5 (OMe-3′), 57.5 (OMe-3″), 62.3 (C-α), 65.2 (C-α′), 68.0 (C-2″), 70.8 (C-5″), 72.7 (C-4″), 81.9 (C-3″), 88.3 (C-2), 101.4 (C-1″), 111.2 (C-2′), 116.7 (C-6), 117.1 (C-4), 119.1 (C-6′), 119.7 (C-5′), 129.5 (C-5), 136.9 (C-3a), 139.2 (C-1′), 141.9 (C-7), 146.3 (C-4′), 146.4 (C-7a), 152.1 (C-3′). Acetylation (Ac_2O–pyridine) of **18** yielded the pentaacetate. ^{1}H NMR ($CDCl_3$): δ1.17 (3H, *d*, H-6″), 1.92 (2H, *m*, H-β), 2.05, 2.07, 2.11, 2.16, 2.30 (5*s*, 5OAc), 2.64 (2H, *t*, H-γ), 3.41 (3H, *s*, OMe-3″), 3.70 (1H, *m*, H-3), 3.84 (3H, *s*, OMe-3′), 3.85 (1H, *dd*, H-3″), 4.00–4.10 (2H, H-4″ and H-5″), 4.08 (2H, *t*, H-α), 4.29 (1H, *dd*, H-α′b), 4.44 (1H, *dd*, H-α′a), 5.38 (1H, *d*, H-1″), 5.52 (1H, *d*, H-2), 5.60 (1H, *dd*, H-2″), 6.80 (1H, *d*, H-4), 6.86 (1H, *dd*, H-6′), 6.88 (1H, *d*, H-6), 6.97 (1H, *d*, H-2′), 7.06 (1H, *d*, H-5′); *J*: 2′,6′ = 2.1, 5′,6′ = 8.2, 2,3 = 6.4, 3,α′a = 5.2, 3,α′b = 8.0, α′a,α′b = 11.3, 4,6 = 1.8, α,β = 6.4, β,γ = 6.7, 1″,2″ = 2.0, 2″,3″ = 3.5, 5″,6″ = 6.3 Hz. Hydrolysis of **18** yielded an aglycone identical (TLC, $[\alpha]_D$ and ^{1}H NMR) to **13**.

Compound **20**. $[\alpha]_D$ − 46° (MeOH; *c* 0.3). UV λ_{max} nm: 277. ^{1}H NMR (CD_3OD): δ1.78 (2H, *m*, H-β), 2.59 (2H, *t*, H-γ), 3.42 (1H, *t*, H-3‴), 3.47 (1H, *dd*, H-2‴), 3.54 (2H, *t*, H-α), 3.57 (1H, *m*, H-4‴), 3.74 (1H, *dd*, H-3b), 3.77 (1H, *s*, OMe-2″), 3.80 (3H, *s*, OMe-3′), 3.83 (1H, *dd*, H-3a), 3.89 (1H, *dd*, H-5‴a), 4.28 (1H, *m*, H-2), 4.84 (1H, *d*, H-1‴), 4.87 (7/8H, *d*, H-$1_{erythro}$), 4.93 (1/8H, *d*, H-1_{threo}), 6.65 (1H, *dd*, H-5″), 6.77 (1H, *d*, H-3″), 6.80 (1H, *d*, H-6″), 6.92 (1H, *dd*, H-6′), 7.01 (1H, *d*, H-5′), 7.08 (1H, *d*, H-2′); *J*: $1_{erythro}$,2 = 5.6, 1_{threo},2 = 5.4, 2,3a = 6.4, 2,3b = 4.0, 3a,3b = 12.2, α,β = 6.4, β,γ = 7.4, 2′,6′ = 2.1, 5′,6′ = 8.2, 3″,5″ = 2.1, 5″,6″ = 8.3, 1‴,2‴ = 7.1, 2‴,3‴ = 8.6, 3‴,4‴ = 8.6, 4‴,5‴a = 5.2, 5‴a,5‴b = 11.6 Hz. The signal from H-5‴b was hidden under the solvent signal at *ca* δ3.30. The signals from H-1 and H-1‴, hidden under the broad hydroxyl peak were visualized by warming the sample to 50°. ^{13}C NMR (CD_3OD): δ32.7 (C-β), 35.5 (C-γ), 56.5 (OMe-2″), 56.6 (OMe-3′), 62.1 (C-3), 62.2 (C-α), 66.8 (C-5‴), 71.0 (C-4‴), 73.9 (C-1), 74.6 (C-2‴), 77.3 (C-3‴), 86.4 (C-2), 103.5 (C-1‴), 112.8 (C-2′), 114.0 (C-6″), 118.0 (C-3″), 119.5 (C-5′), 120.8 (C-6′), 121.8 (C-5″), 138.0

(C-4″), 138.1 (C-1′), 146.9 (C-2″), 147.1 (C-3′), 150.8 (C-1″), 151.8 (C-4′). Acetylation (Ac_2O–pyridine) of **20** yielded the hexaacetate. ^{1}H NMR ($CDCl_3$): δ1.92 (2H, *m*, H-β), 2.02, 2.05, 2.07, 3 × 2.08 (6*s*, 6OAc), 2.61 (2H, *t*, H-γ), 3.40–5.30 (6H, H-1‴–H-5‴), 3.77 (3H, *s*, OMe-2′), 3.81 (3H, *s*, OMe-3′), 4.07 (1H, *t*, H-α), 4.19 (1H, *dd*, H-3b), 4.22 (1H, *dd*, H-3a), 4.60 (1H, *m*, H-2), 6.01 (7/8H, *d*, H-$1_{erythro}$), 6.06 (1/8H, *d*, H-1_{threo}), 6.64 (1H, *dd*, H-5″), 6.68 (1H, *d*, H-3″), 6.75 (1H, *d*, H-6″), 6.93 (1H, *dd*, H-6′), 7.00 (1H, *d*, H-2′), 7.04 (1H, *d*, H-5′); *J*: $1_{erythro}$,2 = 5.2, 1_{threo},2 = 6.7, 2,3a = 4.4, 2,3b = 3.6, 3a,3b = 12.4, α,β = 6.4, β,γ = 7.6, 2′,6′ = 2.1, 5′,6′ = 8.4, 3″,5″ = 2.1, 5″,6″ = 8.0 Hz.

Compound **22**. Mp 105–107°. $[\alpha]_D$ −43° (THF; *c* 0.9). UV λ_{max} nm: 225sh, 282. ^{1}H NMR ($CDCl_3$): δ2.17 (2H, *m*, H-8, H-8′), 2.52 (2H, *dd*, H-7b, H-7′b), 2.59 (2H, *dd*, H-7a, H-7′a), 3.53 (2H, *dd*, H-9b, H-9′b), 3.83 (6H, *s*, OMe-3, OMe-3′), 3.97 (2H, *dd*, H-9a, H-9′a), 6.50 (2H, *d*, H-2, H-2′), 6.58 (2H, *dd*, H-6, H-6′), 6.80 (2H, *d*, H-5, H-5′); *J*: 2,6 = 2′,6′ = 1.8, 5,6 = 5′,6′ = 8.0, 7a,7b = 7′a, 7′b = 13.8, 7a,8 = 7′a,8′ = 6.7, 7b,8 = 7′b,8′ = 7.7, 8,9a = 8′,9′a = 6.7, 8,9b = 8′,9′b = 5.8, 9a,9b = 9′a, 9′b = 8.9 Hz. ^{13}C NMR ($CDCl_3$): δ39.2 (C-7, C-7′), 46.5 (C-8, C-8′), 55.8 (OMe-3, OMe-3′), 73.3 (C-9, C-9′), 111.1 (C-2, C-2′), 114.1 (C-5, C-5′), 121.3 (C-6, C-6′), 132.3 (C-1, C-1′), 143.9 (C-4, C-4′), 146.4 (C-3, C-3′). EI-MS *m/z* (rel. int.): 344 $[M]^+$ (23), 138 (75), 137 (100), 124 (12), 122 (13), 106 (12). Acetylation (Ac_2O–pyridine) of **22** yielded the diacetate. ^{1}H NMR ($CDCl_3$): δ2.22 (2H, *m*, H-8, H-8′), 2 × 2.30 (2*s*, 2OAc), 2.59 (2H, *dd*, H-7b, H-7′b), 2.68 (2H, *dd*, H-7a, H-7′a), 3.55 (2H, *dd*, H-9b, H-9′b), 2 × 3.79 (2*s*, 2OMe), 3.93 (2H, *dd*, H-9a, H-9′a), 6.66 (2H, *dd*, H-6, H-6′), 6.67 (2H, *d*, H-2, H-2′), 6.92 (2H, *d*, H-5, H-5′); *J*: 2,6 = 2′,6′ = 1.8, 5,6 = 5′,6′ = 8.6, 7a,7b = 7′a,7′b = 13.7, 7a,8 = 7′a,8′ = 6.1, 7b,8 = 7′b,8′ = 8.5, 8,9a = 8′, 9′a = 6.7, 8,9b = 8′,9′b = 5.8, 9a,9b = 9′a,9′b = 8.8 Hz.

Compound **26**. $[\alpha]_D$ −43° (MeOH; *c* 0.3). UV λ_{max} nm: 230sh, 280. ^{1}H NMR (CD_3OD): δ2.57 (1H, *dd*, H-4b), 3.07 (1H, *dd*, H-4a), 3.40–3.46 (4H, H-2″-H-5″), 3.72 (1H, *dd*, H-6″b), 3.84 (3H, *s*, OMe-3′), 3.90 (1H, *dd*, H-6″a), 4.00 (1H, *ddd*, H-3), 4.61 (1H, *d*, H-2), 4.84 (1H, *d*, H-1″), 6.02 (1H, *d*, H-6), 6.27 (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 = 7.9, 3,4a = 5.5, 3,4b = 8.5, 4a,4b = 16.5, 6,8 = 2.4, 2′,6′ = 1.8, 5′,6′ = 8.2, 1″,2″ = 7.9, 5″,6″a = 1.6, 5″,6″b = 5.1, 6″a,6″b = 11.9 Hz. The signal from H-1″, hidden under the broad hydroxyl peak was visualized by warming the sample to 50°. ^{13}C NMR (CD_3OD): δ28.9 (C-4), 56.4 (OMe-3′), 62.6 (C-6″), 68.7 (C-3), 71.3 (C-4″), 74.9 (C-2″), 78.2 (C-3″), 78.3 (C-5″), 83.1 (C-2), 96.9 (C-8), 98.1 (C-6), 102.5 (C-1″), 103.5 (C-4a), 111.9 (C-2′), 116.0 (C-5′), 121.3 (C-6′), 131.9 (C-1′), 147.5 (C-4′), 148.9 (C-3′), 156.7 (C-7), 157.9 (C-5), 158.1 (C-8a). Acetylation (Ac_2O–pyridine) of **26** yielded the heptaacetate. ^{1}H NMR ($CDCl_3$): δ1.97, 1.98, 2.02, 2.05, 2.10, 2.28, 2.30 (7*s*, 7OAc), 2.72 (2H, *d*, H-4a, H-4b), 3.80 (3H, *s*, OMe-3′), 3.89 (1H, *ddd*, H-5″), 4.18 (1H, *dd*, H-6″b), 4.27 (1H, *dd*, H-6″a), 4.31 (1H, *dt*, H-3), 5.03–5.09 (2H, *m*, H-2″, H-4″), 5.14 (1H, *dd*, H-3″), 5.27 (1H, *d*, H-1″), 5.28 (1H, *d*, H-2), 6.40 (1H, *d*, H-6), 6.50 (1H, *d*, H-8), 6.90 (1H, *dd*, H-6′), 6.95 (1H, *d*, H-2′), 6.99 (1H, *d*, H-5′); *J*: 2,3 = 6.2, 3,4 = 6.5, 6,8 = 2.1, 2′,6′ = 1.8, 5′,6′ = 8.1, 1″,2″ = 8.5, 2″,3″ = 9.9, 5″,6″a = 2.6, 5″,6″b = 5.8, 6″a, 6″b = 12.4 Hz. Aglycone of **26**. ^{1}H NMR (CD_3OD): δ2.51 (1H, *dd*, H-4b), 2.89 (1H, *dd*, H-4a), 3.84 (3H, *s*, OMe-3′), 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.

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