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SYMPLOCOSIDE, A FLAVANOL GLYCOSIDE FROM *SYMPLOCOS UNIFLORA*

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Key Word Index—*Symplocos uniflora*; Symplocaceae; flavanol glycoside; symplocoside; symplocosidin; 3′-*O*-methyl-(−)-epicatechin 7-*O*-β-D-glucoside.

Abstract—A new flavanol glycoside, symplocoside, was isolated from the MeOH extract of the stem bark of *Symplocos uniflora* and its constitution and conformation were elucidated by means of MS, ^{1}H and ^{13}C NMR spectroscopy as (2*R*:3*R*)-7-*O*-β-D-glucopyranosyl-3′-*O*-methyl-(−)-epicatechin.

INTRODUCTION

Symplocos uniflora (Pohl) Benth. (syn: *Stemmatosiphon uniflorum* (Pohl)) belongs to the species group *Ciponimastrum* Brand, which contains *ca* 40 species and is restricted to the tropics of the New World; it belongs to the subgenus *Eusymplocos* Brand [1, 2]. It is a shrub or small tree found in the middle east of Brazil (Minas Gerais, Morretes-Parana) [3]. In Brazilian popular medicine, this drug plant is said to be of some importance; its popular name is 'Maria mola' (soft Maria) (Hatschbach, G., Kramer, K. and Wasicky, K., private communication). So far, only saponins, namely triterpenoid saponins with 4–5 sugar units are known constituents of this plant [1, 4].

RESULTS AND DISCUSSION

From the MeOH extract of the stem bark of *S. uniflora*, a light- and oxidation-sensitive flavanol glycoside, $C_{22}H_{26}O_{11}$ (symplocoside **1**) was obtained in a crystalline form by repeated CC under protection from light (Al-foil); under N_2 and in the dark, it is stable for a longer time without decomposition. The peracetate **3** and the permethyl ether **5** are more stable. Mild enzymatic cleavage of **1** with β-glucosidase or the mixed enzyme EL 27-67 yielded a carbohydrate moiety and an aglycone **2**. The sugar was identified by PC [5] and after silylation by GLC [6] as D-glucose. The crystalline aglycone, $C_{16}H_{16}O_6$ (symplocosidin **2**) was converted with diazomethane after purification by CC into the methyl ether **6**; a comparison of the physical data and the ^{1}H and ^{13}C NMR spectral data with the literature [7] identified **6** as (−)-epicatechin-tetramethyl ether.

The position of the original methoxy group could be located by a ^{1}H [^{1}H] double-resonance experiment of the glycoside **1** in DMSO-d_6 [8]: therefore the pronounced nuclear Overhauser enhancement (NOE) of an aromatic proton *ortho* to a methoxy group was used by irradiating at δ 3.75 ppm with a transmitter power of 0.5×10^{-7} W upon the signal of the methoxy group (ring B), while observing the intensities of the B-ring protons (Fig. 1). The integral

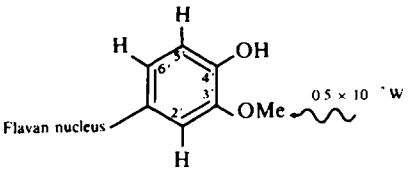


Fig. 1

intensities of the protons H-5′ (δ 6.79 ppm, J_{ortho} = 8.21 Hz) and H-6′ (δ 6.96 ppm, J_{ortho} = 8.21 Hz, J_{meta} = 1.91 Hz) remained fairly constant whereas the integral intensity of the only *meta*-coupling proton H-2′ (δ 7.18 ppm, J_{meta} = 1.91 Hz) was enhanced by *ca* 27%; thus, the position of the methoxy group could be confirmed, in agreement with the literature [8], to be on C-3′. If it had been on C-4′ of ring B, the integral intensity of the only *ortho*-coupled proton H-5′ would have increased.

Therefore, the constitution and conformation of the aglycone **2** was established as (2*R*:3*R*)-3′-*O*-methyl-(−)-epicatechin [9]; it is the first time that **2** has been found as naturally occurring aglycone in a flavanoid glycoside of known constitution [10]. The racemate, (±)-3′-*O*-methyl-epicatechin, has already been prepared synthetically by $NaBH_4$ reduction of peonidin [11].

The sugar was shown to be attached to a phenolic OH group by means of ^{1}H NMR spectroscopy in DMSO-d_6 of **1** (2 phenolic OH groups at δ 8.84 and 9.13 ppm) and of **2** (3 phenolic OH groups at δ 8.83, 8.89 and 9.11 ppm); the localization of the carbohydrate moiety either at ring A (C-5-OH or C-7-OH) or at ring B (C-4′-OH) could be determined by methylation of **1** with diazomethane, enzymatic cleavage of the methylated glycoside **4** with the enzyme mixture EL 27-67, acetylation of the partially methylated aglycone **7** to yield **8** and MS investigation of **8**: high resolution MS of the trimethyl-diacetyl-aglycone **8** led to the retro-Diels–Alder fragments A′ (*m/e* = 195.0661 ≙ $C_{10}H_{11}O_4$) and B′ (*m/e* = 222.0890 ≙ $C_{12}H_{14}O_4$) (Fig. 2).

Thus, the sugar must be attached to ring A either at the 5- or 7-positions. These possibilities could be distinguished

Table 1. ^{13}C NMR data (22.63 MHz) of **1** (CD_3OD) and **3** ($CDCl_3$), TMS_{int}.

C-atom	δ (ppm) 1	3	Multiplicity (off-resonance)	C–H coupling constants (Hz) 1	3
C-2	79.98	77.35	doublet		
C-3	67.29	68.49	doublet		
C-4	29.39	26.19	triplet	—	
C-4a	102.64	107.27	singlet	$^3J_{CH}$ = 5.9 (H-6/)	$^2J_{CH}$ = 4.4 ($H\text{-}4_{eq;ax}$)
				$^3J_{CH}$ = 5.4 (H-8)	$^3J_{CH}$ = 9.1 (H-6/8)
C-5	157.12	150.15	singlet	$^3J_{CH}$ = 5.0 ($H\text{-}4_{ax}$/)	$^3J_{CH}$ = 5.0 ($H\text{-}4_{ax}$/)
				$^3J_{CH}$ = 4.8 ($H\text{-}4_{eq}$)	$^3J_{CH}$ = 4.8 ($H\text{-}4_{eq}$)
C-6	97.07	101.83	doublet	$^1J_{CH}$ = 159.8	$^1J_{CH}$ = 164.8
				$^3J_{CH}$ = 4.4 (H-8)	$^3J_{CH}$ = 5.1 (H-8)
C-7	157.79	155.82	singlet	$^2J_{CH}$ = 3.7 (H-6/)	$^2J_{CH}$ = 2.7 (H-6/)
				$^2J_{CH}$ = 3.7 (H-8)	$^2J_{CH}$ = 2.9 (H-8)
C-8	98.59	105.65	doublet	$^1J_{CH}$ = 160.1	$^1J_{CH}$ = 164.8
				$^3J_{CH}$ = 4.5 (H-6)	$^3J_{CH}$ = 4.4 (H-6)
C-8a	158.41	155.82	singlet	not resolved	$^2J_{CH}$ = 3.5 (H-8)
C-1′	132.13	136.24	singlet	$^2J_{CH}$ = 3.0 (H-2′/H-6′)	$^2J_{CH}$ = 3.7 (H-2′/H-6′)
				$^3J_{CH}$ = 6.1 (H-5′)	$^3J_{CH}$ = 5.9 (H-5′)
C-2′	111.90	110.89	doublet	$^1J_{CH}$ = 157.4	$^1J_{CH}$ = 159.2
				$^3J_{CH}$ = 5.9 (H-6′)	overlapped
C-3′	148.63	151.13	singlet	$^2J_{CH}$ = 4.0 (H-2′)	$^2J_{CH}$ = 3.7 (H-2′)
				$^3J_{CH}$ = 8.1 (H-5′)	$^3J_{CH}$ = 7.4 (H-5′)
C-4′	146.98	139.73	singlet	$^3J_{CH}$ = 8.1 (H-2′/)	$^3J_{CH}$ = 7.4 (H-2′/)
				$^3J_{CH}$ = 7.3 (H-6′)	$^3J_{CH}$ = 5.8 (H-6′)
C-5′	115.78	122.71	doublet	$^1J_{CH}$ = 158.2	$^1J_{CH}$ = 162.6
C-6′	120.64	118.79	doublet	$^1J_{CH}$ = 159.9	$^1J_{CH}$ = 163.4
				$^2J_{CH}$ = 3.6 (H-5′)	$^2J_{CH}$ = 3.7 (H-5′)
				$^3J_{CH}$ = 7.2 (H-2′)	$^3J_{CH}$ = 7.4 (H-2′)
C-1″	102.45	99.40	doublet	$^1J_{CH}$ = 161.1	$^1J_{CH}$ = 164.1
					$^2J_{CH}$ = 2.5 (H-2″)
C-2″	74.86	70.95	doublet	—	—
C-3″	78.01[a]	72.63[a]	doublet	—	—
C-4″	71.24	67.42	doublet	—	—
C-5″	78.14[a]	72.28[a]	doublet	—	—
C-6″	62.50	62.11	triplet	—	—
—OMe	56.48	56.03	quartet	$^1J_{CH}$ = 144.3	$^1J_{CH}$ = 145.1
—Me (acetyl)	—	20.45–21.10	quartets	—	—
>C=O (acetyl)	—	168.93–170.55	singlets	—	—

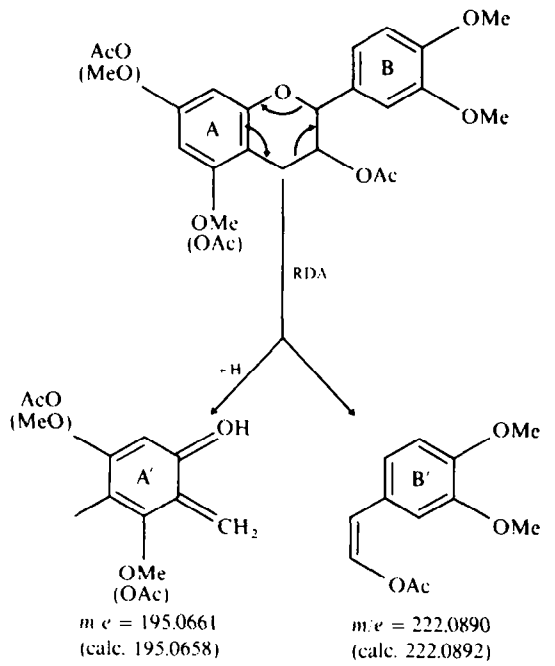


Fig. 2

by a comparison of the ^{1}H NMR spectra of **4** and **8**: on acetylation of **7** a symmetric downfield shift of ~0.15 ppm (calc. 0.29 ppm) [12, 13] resulted for the protons H-6 and H-8, suggesting the acetate position to be on C-7, where both protons H-6 and H-8 were equally deshielded. Thus the glucose is at C-7-OH.

From **1** and **3**, ^{13}C [^{1}H] NMR spectra were recorded; the signals of the noise-decoupled ^{13}C NMR spectra could be unequivocally assigned based on their chemical shifts, calculated by increment estimating, and on their multiplicities, found in the off-resonance-decoupled ^{13}C NMR spectra. The signals of the ^{1}H-coupled-^{13}C NMR spectra were completely assigned by the C-H coupling constants (> 90 ppm). (Table 1). Following more recent work in the field of ^{13}C NMR spectroscopy [8, 15], the signals (*ca* 95 ppm) with the smaller shifts against TMS were assigned to C-6 although increment estimating and other investigations gave different results [16].

The chemical shifts of δ 102.45 (**1**) and 99.4 ppm (**3**) for the anomeric C-atom clearly indicate a β-linkage of the carbohydrate moiety [14], corresponding to the above enzymatic results. Consequently, the constitution and conformation of the glycoside **1** is established in agreement with [10, 17] as: (2*R*:3*R*)-7-*O*-β-D-glucopyranosyl-3'-*O*-methyl-(−)-epicatechin or (2*R*,*cis*)-2H-[1]-benzopyrane-3,4-dihydro-3,5-dihydroxy-7-*O*-β-D-glucopyranosyl-2 [4-hydroxy-3-methoxy-phenyl] (Fig. 3).

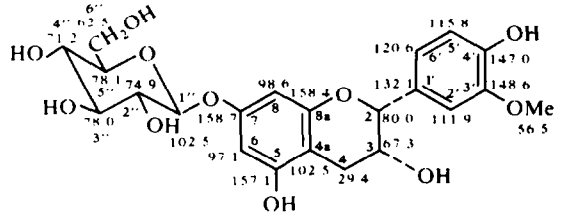
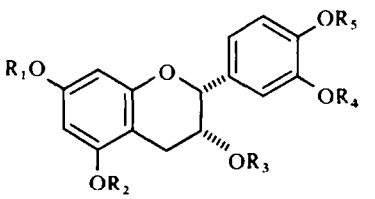


Fig. 3

The glycoside **1** belongs to the class of the naturally occurring flavan-3-ols (11 members known [10]) namely to the subdivision of the flavan-3-ol monomethyl ethers (one member known [18]); it belongs also to the class of the very rare and unusual flavanol glycosides of which only three members were isolated or synthesized previously [19]. It is not clear how far the glycoside **1** has structural analogies to an earlier isolated glycoside with the same elementary composition because no physical data were available [20]. According to investigations of Willstätter *et al.* [21, 22] and of Marini-Bettòlo [18], these earlier results were inconclusive. Thus, **1** is the second naturally occurring flavanol monomethyl ether, whose constitution and conformation has been fully characterized; the first such ether was isolated by Marini-Bettòlo in 1967 and identified as (2*R*:3*R*)-4'-*O*-methyl-(−)-epigallocatechin [10, 18]. We propose the name symplocoside for **1** and symplocosidin for the aglycone **2**.



	1	2	3	4	5	6	7	8
R_1	β-Glc	H	β-Glc. Ac_4	β-Glc	β-Glc. Me_4	Me	H	Ac
R_2	H	H	Ac	Me	Me	Me	Me	Me
R_3	H	H	Ac	H	Me	H	H	Ac
R_4	Me	Me	Me	Me	Me	Me	Me	Me
R_5	H	H	Ac	Me	Me	Me	Me	Me

EXPERIMENTAL

Mps are uncorr.; GLC on chromatograph F 22 with Integrator D 2, a Kienzle digital plotter and a FID as detecting unit; the column was a 2 m glass column (ϕ 2.4 mm) containing 3% OV 101 on Gaschrom Q (80–100 mesh). Carrier gas was N_2 (30 ml/min) at 155°. ^{1}H NMR (90 MHz, PFT, TMS_{int}) and ^{13}C NMR (22.63 MHz, PFT, $TMS_{int.}$) spectra were recorded with a WH 90 PFT (Bruker-physik) and MS (DE, 180°C, 70 eV) on a MS-50 (A.E.I.) with a DS-50 data unit (Data General). TLC was run on Si gel sheets F 1500 LS 254 (Schleicher & Schüll) using 40% H_2SO_4 as detecting reagent [23]; PC was on Selecta No. 2043b Mgl (Schleicher & Schüll. CC was run on sieved Si gel WOELM (0.063–0.1 mn ϕ) and unisieved Si gel Hermann. The following solvents were used A, $CHCl_3$–MeOH–H_2O(65:20:2); B, $CHCl_3$–MeOH–H_2O (80:18:2); C, $CHCl_3$–MeOH–H_2O (65:45:12); D, EtOAc–Me_2CO–H_2O (25:5:1); E, $CHCl_3$–Me_2CO (100:1–10:1); F, $CHCl_3$–MeOH–H_2O (120:12:1); G, Cyclohexane–Me_2CO (7:1–5:1); H, Py–EtOAc–H_2O (100:360:115) [5]. The enzyme mixture EL 27-67 (Röhm & Hass), containing β-glucosidase, β-galactosidase and α-rhamnosidase as well as pure β-glucosidase (Roth) were used for fermentative cleavage.

Isolation of the glycoside. Air-dried stem bark of *Symplocos uniflora* (1.5 kg) was collected in April 1976 by the Laboratórios Gemballa Ltda., Rio do Sul, Brazil. The powdered drug was

extracted at room temp. in an ultraturrax with 10 l. MeOH (× 6) and concd at 40° to yield 170 g syrupy extract, which was dissolved in 500 ml H_2O and extracted with *n*-BuOH (250 ml × 6) to give 64 g. The *n*-BuOH extract was submitted in three portions to CC on Si gel with solvent A to yield 11.2 g of crude glycoside and *ca* 4 of foaming and haemolytic fractions (saponins); the glycosidic fractions were purified several times under protection from light (alumina foil) by CC with the solvents B and D to give 940 mg microcrystalline colourless powder (**1**), that decomposes in the presence of light or air-oxygen turning its colour to red; mp: 171 174° (dec.), $R_f = 0.11$(D). $[\alpha]_{Na-589}^{20} -77.3°$, $[\alpha]_{Hg-578}^{20} -81.1°$ ($c = 2.700$ in EtOH). UV nm: λ_{max}^{MeCN} (log ε): 278 (3.66); 227 (4.26); 204 (4.91). ^{1}H NMR (DMSO-d_6) (ppm): δ 2.76 (2 H, *s*, H-4); 3.14–5.18 (sugar, 11 H, overlapped); 3.75 (3 H, *s*, OMe); 4.27 (1 H, *m*, H-3); 4.73 (1 H, *d*, ^4J = 4.1 Hz, C-3-OH*); 4.81 (1 H, *br*, H-2); 5.11 (1 H, *m*, H-1 Glc); 6.07 (1 H, *d*, ^4J = 2.34 Hz, H-6); 6.31 (1 H, *d*, ^4J = 2.34 Hz, H-8); 6.79 (1 H, *d*, ^3J = 8.21 Hz, H-5'); 6.96 (1 H, *dd*, ^4J = 1.91 Hz, ^3J = 8.21 Hz, H-6'); 7.18 (1 H, *d*, ^4J = 1.91 Hz, H-2'); 8.84 (1 H, *s*, C-5-OH*); 9.13 (1 H, *s*, C-4'-OH*). * = exchangeable with D_2O. ^{13}C NMR ($^{12}CD_3OD$): see Table 1. EI-MS(*m/e*): no M^+; 328; 304 (M − Glc); 286 (aglycone − H_2O); 167; 166; 151; 139; 137 (100 %, RDA, A'-H); 123; 73.

Acetylation of **1**. 500 mg of **1** were acetylated with Py-Ac_2O [24], purified by CC on Si gel (solvent E) and recrystallized from petrol-Me_2CO: colourless needles (364 mg) (**3**); mp: 198–201°, $R_f = 0.41$ (E). $[\alpha]_{Na-589}^{20} -53.5°$, $[\alpha]_{Hg-578}^{20} -55.9°$ ($c = 1.032$ in $CHCl_3$). ^{1}H NMR ($CDCl_3$) (ppm): δ 1.89 (3 H, *s*, C-3-acetyl-Me); 2.02 (6 H, *s*), 2.05 (3 H, *s*), 2.09 (3 H, *s*, 4x sugar-acetyl-Me); 2.29 (3 H, *s*, C-5-acetyl-Me); 2.31 (3 H, *s*, C-4'-acetyl-Me); 2.77 (1 H, *m*, H-5 Glc); 2.87 (1 H, *d*, ^3J = 2.0 Hz, H-4$_{eq}$); 2.95 (1 H, *d*, ^3J = 4.4 Hz, H-4$_{ax}$); 3.84 (3 H, *s*, OMe); 4.23 (2 H, *m*, -CH_2-Glc); 5.00 5.53 (6 H, *m*, H-3, H-2, H-1 Glc, H-2 Glc, H-3 Glc, H-4 Glc); 6.52 (1 H, *d*, ^4J = 2.05 Hz, H-6); 6.62 (1 H, *d*, ^4J = 2.20 Hz, H-8); 7.05 (1 H, *d*, ^3J = 8.0 Hz, H-5'); 7.15 (1 H, *d*, ^3J = 8.0 Hz, ^4J = not res., H-6'); 7.29 (1 H, *s*, not res., H-2'). ^{13}C NMR ($CDCl_3$): see Table 1. High-resolution MS: $C_{36}H_{40}O_{18}$ *m/e* = 760.2171 (calc. 760.2215). CH-analysis: $C_{36}H_{40}O_{18}$. Found: C, 56.88; H, 5.27. Calc.: C, 56.84; H, 5.30 %.

Permethylation of **1**. 51 mg of **1** were permethylated (Hakomori's method [25]) purified by CC on Si gel (solvent E) and recrystallized from Me_2CO-d_6: almost colourless needles (29 mg) (**5**); mp: 150 153°, $R_f = 0.21$ (G). $[\alpha]_{Na-589}^{20} -71.6°$, $[\alpha]_{Hg-578}^{20} -75.2°$ ($c = 1.04$ p4 in $CHCl_3$). ^{1}H NMR ($CDCl_3$) (ppm): δ 2.88 (1 H, *d*, ^3J = 3.81 Hz, H-4$_{eq}$); 2.98 (1H, *d*, ^3J = 3.08 Hz, H-4$_{ax}$); 3.11–4.00 (7 H, *m*, H-2 Glc, H-3 Glc, H-4 Glc, H-5 Glc, -CH_2Glc, H-3, overlapped by 8x OMe); 3.27 (3 H, *s*, C-3-OMe); 3.38 (3 H, *s*), 3.54 (3 H, *s*), 3.64 (3 H, *s*), 3.66 (3 H, *s*, 4x sugar-OMe); 3.73 (3 H, *s*, C-5-OMe); 3.89 (3 H, *s*, C-3'-OMe)[a]; 3.90 (3 H, *s*, C-4'-OMe)[a]; 4.80 (1 H, *m*, H-1 Glc); 4.95 (1 H, *s*(*br*), $\Delta\nu_{1/2}$ = 3.4 Hz, H-2); 6.27 (2 H, *s*, not res., H-6, H-8); 6.85 (1 H, *d*, ^3J = 8.5 Hz, H-5'); 6.99 (1 H, *dd*, ^3J = 8.50 Hz, ^4J = 1.76 Hz, H-6'); 7.15 (1 H, *d*, ^4J = 1.76 Hz, H-2'). ^{13}C NMR ($^{12}CDCl_3$) (ppm): δ 24.15 (sec, C-4); 55.38 (prim, C-5-OMe); 55.96 (2x prim, C-3'-OMe, C-4'-OMe); 57.55 (prim, C-3-OMe); 59.33 (prim), 60.43 (prim), 60.75 (prim), 61.01 (prim, 4x sugar-OMe); 71.08 (sec, C-6 Glc); 74.80 (tert, C-4 Glc)[a]; 75.35 (tert, C-3)[a]; 78.23 (tert, C-2)[b]; 79.21 (tert, C-2 Glc)[b]; 83.64 (tert, C-3 Glc)[c]; 86.68 (tert, C-5 Glc)[c]; 95.58 (quart, C-6)[d]; 95.71 (quart, C-8)[d]; 101.12 (tert, C-1 Glc); 101.93 (quart, C-4a); 110.73 (tert, C-2')[e]; 110.80 (tert, C-5')[e]; 119.41 (tert, C-6'); 131.45 (quart, C-1'); 148.83 (2x quart, C-3' C-4'); 155.66 (quart, C-5); 156.91 (quart, C-7); 159.51 (quart, C-8a). High-resolution MS: $C_{29}H_{40}O_{11}$ *m/e* = 564.2575 (calc. 564.2570).

Hydrolysis of **5**. **5** (10 mg) was hydrolysed with 5 % methanolic HCl-2 N HCl and worked up as usual [4]; only 2,3,4,6-tetra-*O*-methylglucose could be detected by TLC (solvent E).

Methylation of **1**. **1** (160 mg) was methylated at −5° with ethereal CH_2N_2 λ [4], pirified by CC on Si gel (solvent F) and recrystallized from MeOH-d_4: colourless needles (63 mg) (**4**); mp: 216 220° (dec.), $R_f = 0.16$ (E). $[\alpha]_{Na-589}^{20} -22.1°$, $[\alpha]_{Hg-578}^{20} -25.7°$ ($c = 1.03$ in MeOH). ^{1}H NMR (CD_3OD–Me_2CO-d_6 1:1) (ppm): δ 2.83 (2 H, *d*, ^3J = 4.0 Hz, H-4$_{eq}$, H-4$_{ax}$); 3.43 (2 H, *s*, -CH_2-Glc); 3.74 (3 H, *s*, C-5-OMe); 3.83 (3 H, *s*, C-3'-OMe)[a]; 3.84 (3 H, *s*, C-4'-OMe)[a]; 3.08–4.80 (5 H, *m*, overlapped, H-3, H-2 Glc, H-3 Glc, H-4 Glc, H-5 Glc); 4.90 (1 H, *m*, overlapped, H-1 Glc); 4.94 (1 H, *s*(*br*), $\Delta\nu_{1/2}$ = 2.8 Hz, H-2); 6.21 (1 H, *d*, ^4J = 2.49 Hz, H-6); 6.43 (1 H, *d*, ^4J = 2.34 Hz, H-8); 6.92 (1 H, *d*, ^3J = 8.20 Hz, H-5'); 7.05 (1 H, *dd*, ^3J = 8.20 Hz, ^4J = 1.80 Hz, H-6'); 7.18 (1 H, *s*, ^4J = not res.). High-resolution MS: $C_{24}H_{30}O_{11}$ *m/e* = 494.1779 (calc. 494.1788).

Enzymatic cleavage of **4**. **4** (40 mg) was suspended in H_2O with 5 mg of the enzyme mixture EL 27–67 and 5 drops xylene; the reaction mixture was stirred at room temp. under protection from light. Every 12 hr, a small quantity of fresh enzyme was added, and after 7 days the cleavage was complete. 40 ml MeOH were then added. The mixture was refluxed for 30 min and filtered after cooling; the filtrate was concd and separated between H_2O and *n*-BuOH. From the H_2O layer only D-glucose could be detected (TLC with the solvent C). The organic layer gave 25 mg of partially methylated aglycone **7**.

Acetylation of **7**. **7** (10 mg) was acetylated with Py-Ac_2O [24] and purified by CC on Si gel (solvent K): 1.5 mg of a colourless oil that does not crystallize (**8**). $R_f = 0.19$ (F), 0.12 (G). ^{1}H NMR ($CDCl_3$) (ppm): δ 1.93 (3 H, *s*, C-3-acetyl-Me); 2.30 (3 H, *s*, C-7-acetyl-Me); 2.82 (1 H, *d*, ^3J = 2.6 Hz, H-4$_{eq}$, partially overlapped); 2.87 (1 H, *d*, ^3J = 5.40 Hz, H-4$_{ax}$, partially overlapped); 3.78 (3 H, *s*, C-5-OMe); 3.89 (3 H, *s*, C-3'-OMe)[a]; 3.90 (3 H, *s*, C-4'-OMe)[a]; 5.05 (1 H, *s*(*br*), $\Delta\nu_{1/2}$ = 3.0 Hz, H-2); 5.39 (1 H, *m*, not res., H-3); 6.33 (1 H, *d*, ^4J = 1.80 Hz, H-6); 6.48 (1 H, *d*, ^4J = 1.80 Hz, H-8); 6.80 6.93 (2 H, *m*, not res., H-5', H-6'); 7.02 (1 H, *s*, not res., H-2'). For a comparison with **4**, a ^{1}H NMR spectrum was run in CD_3OD Me_2CO-d_6 1:1, too: 6.37 (1 H, *d*, ^4J = 2.20 Hz, H-6); 6.57 (1 H, *d*, ^4J = 2.20 Hz, H-8). High-resolution MS: $C_{22}H_{24}O_8$ *m/e* = 416.1465 (calc. 416.1471).

Enzymatic cleavage of **1**. **1** (600 mg) was incubated in a breeding box for 40 hr at 39° with 20 mg enzyme mixture EL 27–67 and 10 drops of xylene in 100 ml water and worked up as above. (An analogous cleavage with pure β-glucosidase was successful too.) From the H_2O layer, only D-glucose was detected by comparison with authentic specimens by PC [5] and by GLC after transformation into the 1-*O*-methyl-pertrimethylsilyl ether [4, 6]; the organic layer (370 mg of crude aglycone **2**) was purified by CC on Si gel (solvent F) and recrystallized from CD_3OD–$CDCl_3$: colourless prisms (230 mg) (**2**); mp: 259 264°, $R_f = 0.21$ (F). $[\alpha]_{Na-589}^{20} -3.1°$, $[\alpha]_{Hg-578}^{20} -5.7°$ ($c = 1.016$ in MeOH). ^{1}H NMR (DMSO-d_6) (ppm): δ 2.64 (1 H, *d*, ^3J = 4.40 Hz, H-4$_{eq}$, partially overlapped by DMSO); 3.17 (1 H, *d*, ^4J = 5.13 Hz, H-4$_{ax}$); 3.74 (3 H, *s*, OMe); 4.07 (1 H, *m*, H-3); 4.67 (1 H, *d*, ^4J = 5.13 Hz, C-3-OH*); 4.79 (1 H, *s*(*br*), $\Delta\nu_{1/2}$ = 3.4 Hz, H-2); 5.74 (1 H, *d*, ^4J = 2.20 Hz, H-6); 5.90 (1 H, *d*, ^4J = 2.20 Hz, H-8); 6.71 (1 H, *d*, ^3J = 8.06 Hz, H-5'); 6.85 (1 H, *dd*, ^3J = 8.06 Hz, ^4J = 1.46 Hz, H-6'); 7.03 (1 H, ^3J = 1.46 Hz, H-2'); 8.83 (1 H, *s*, C-5-OH*); 8.89 (1 H, *s*, C-7-OH*); 9.11 (1 H, *s*, C-4'-OH*). * = exchangeable with D_2O. EI-MS (*m/e*): 305 (4.6 %, M + H); 304 (25.5, M^+); 286 (1.6); 167 (21.1); 166 (99.4); 151 (7.2); 139 (100, RDA, A' + H); 138 (58.5); 137 (50.5); 123 (5.9); 106 (2.2); 95 (3.7); 77 (2.5). High-resolution MS: $C_{16}H_{16}O_6$ *m/e* = 304.0947 (calc. 304.0947).

Methylation of **2**. **2** (160 mg) was methylated with ethereal CH_2N_2 and purified by CC on Si gel (solvent E): 74 mg of a light yellow oil, part of which crystallized from Me_2CO-d_6 $CDCl_3$: colourless needles (23 mg) (**7**): mp: 154–156°, $R_f = 0.91$ (F).

$[\alpha]_{Na\cdot 589}^{20} -59.2°$, $[\alpha]_{Hg\cdot 578}^{20} -62.1°$ ($c = 1.667$ in $CHCl_3$). High-resolution MS: $C_{19}H_{22}O_6$ *m/e* = 346.1449 (calc. 346.1416). The spectral data agree with those in lit. [7, 8, 15]

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