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VEBRASIDE, AN IRIDOID GLUCOSIDE FROM *VERBENA BRASILIENSIS*

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Key Word Index—*Verbena brasiliensis*; Verbenaceae; iridoids; vebraside; brasoside.

Abstract—*Verbena brasiliensis* contains besides brasoside a novel 3-*O*-glucopyranosyl-3,4-dihydroiridoid, vebraside, which was fully characterized by spectroscopic data (UV, IR, MS, ^{1}H and ^{13}C NMR) and chemical analysis.

INTRODUCTION

Earlier investigations of *Verbena brasiliensis* Vell. led to the isolation of brasoside (**5**) [1]. We now report the isolation and structure elucidation of the iridoid glucoside, vebraside (**1**) from a complex iridoid-containing fraction of *V. brasiliensis*.

RESULTS AND DISCUSSION

Verbraside (**1**) showed one UV maximum at 187 nm and IR peaks at 3600–3200 and 1761 cm^{-1} assignable to hydroxyl groups and a γ-lactone [2]. Its FDMS spectrum contained peaks at *m/z* 399, 203 and 196 due to $[M + Na]^+$ $[hexose + Na]^+$ and $[M - hexose]^+$ indicating a molecular formula of $C_{16}H_{24}O_{10}$. On acid hydrolysis, it gave one mol glucose and many products due to the decomposition of the aglucone. On alkaline hydrolysis, it gave the same products together with the aglucone **2**. Enzymatic hydrolysis with β-glucosidase, however, afforded one mol glucose and exclusively **2** which was isolated for spectroscopic analysis.

The ^{13}C NMR data (Table 1) of **1** were similar to those of brasoside (**5**) [3] and cistanin (**8**) [4]. The data for **1** showed good agreement with those of **5** concerning the values of the cyclopentane and lactone parts of the molecules and agreed with those of **8** concerning the chemical shifts of the tetrahydropyran moiety. Structure **1**, therefore, had to be a dihydroiridoid lactone glucoside. Structure **1** for vebraside was confirmed by ^{1}H NMR analysis (Table 2): double resonance experiments proved the β-configuration of the glucopyranosyl moiety ($J_{1',2}$ = 7.5 Hz) and allowed the complete assignment of the protons of the aglucone part (Table 2 and 3). The unusual coupling of H-4 with the acetalic proton at δ 6.22 and the positive NOEs between H-3 and H-4 as well as H-1′ is only compatible with a glucosyloxy group at C-3. The methyleneoxide proton at δ 4.4 was assigned to H-1β since there is a positive NOE as well as a coupling (3.5 Hz) between this proton and H-9. The other methyleneoxide proton at δ 3.7 was assigned to H-1α: it coupled exclusively with the geminal H-1β (13 Hz) and showed no NOE on H-9 because of the *trans* relationship between H-1α and H-9. The coupling constant $J_{5,9}$ = 9.5 Hz indicated the normal *cis*-junction of the cyclopentane and pyran rings [5]. The *cis* relationship of H-4, H-5, H-6 and H-9 was shown by the positive NOEs between them (Table 3). Assuming that **1** has the same absolute configuration as the other known iridoid glucosides at C-5 and C-9 these NOEs also proved the β-position of H-4 and H-6. The γ-lactone, therefore, is on the α-site as it is in analogous iridoids [6]. The coupling constant $J_{4,3}$ = 1.0 Hz suggested an α-position of H-3 and the lack of NOE between H-8 and H-9 together with the positive NOE between H_3-10 and H-9 proved the β-position of the methyl group at C-8. The coupling constant $J_{7,6} < 1$ Hz and the lack of NOE between H-7 and H-9 demonstrated that H-7 is on the α-site. This result is in good agreement with the fact that the C-10 signal appears at very high field (δ 10.9) in the ^{13}C NMR spectrum of **1**, which is characteristic for 7β-OH–8β-Me iridoids [7]. The coupling constants $J_{9,10} < 1$ Hz, $J_{9,1\beta}$ = 3.5 Hz and $J_{8,9}$ = 12.0 Hz are compatible with a $^5C_2$-conformation of the pyran ring and a $V_8/^7T_8$-conformation for the cyclopentane moiety. The unusual methyleneoxide structure at C-1 was confirmed by the mass spectra (Fig. 1) of the aglucone **2** and its tetramethylsilane derivative (**3**): Fragments B and C indicated that there is no substituent at the 'lower part' (C-1, C-8, C-9, C-10) of **2** [8, 9]. Thus the structure and relative configuration of **1** is established. We propose the name vebraside for this new compound.

Methanolysis of **1** yielded β-D-glucose and compounds **4** and **6**. The mass spectrum of **4** showed a molecular peak at *m/z* 228 ($C_{11}H_{16}O_5$) and analogous signals as observed in the spectrum of **2** except for the $[M - 17]^+$ peak and fragment B (Fig. 1). Since the ^{1}H coupling constants and NOEs as well as the ^{13}C NMR spectrum of **4** were very similar to those of the aglucone part of **1** (see Tables 1 and 2) **4** was shown to be the methylacetal of **2**. The mass spectrum of **6** also showed a molecular ion at *m/z* 228. But the ^{1}H NMR spectrum showed a doublet of an olefinic proton (δ 7.78) and a sharp singlet of a methylester (δ 3.69) which were not present in the spectrum of **1**. Homonuclear ^{1}H NMR decoupling experiments and NOE data allowed the assignment of all the signals (Table 2) and proved structure **6** for this reaction product. The coupling constants are compatible with a $V_8/^7T_8$-

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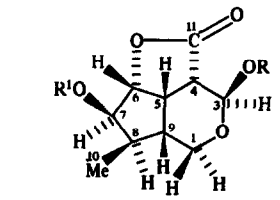
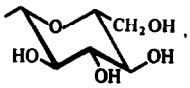
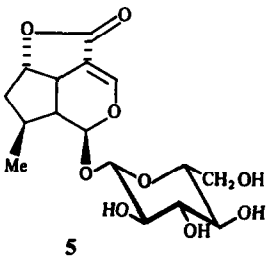


1 R =  , R^1 = H

2 R = R^1 = H

3 R = R^1 = —$Si(Me)_3$

4 R = Me, R^1 = H



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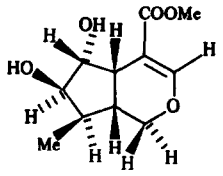

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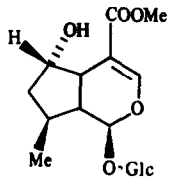

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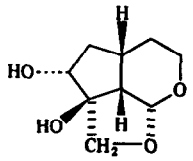

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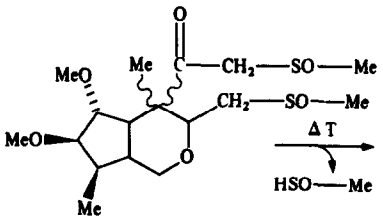

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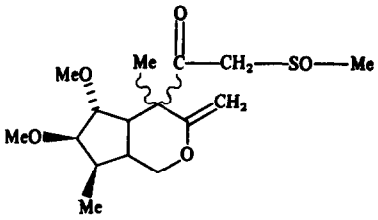

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conformation of the cyclopentane and a $^5B_0$-conformation of the pyran ring.

The unusually facile cleavage of the glycosidic bond of 1 during hydrolysis and methanolysis under basic conditions has not been observed with iridoid-1-*O*-glucosides, and the reaction proceeded with overall retention of configuration. A direct substitution at C-3, therefore, seems rather unlikely. The observation, that H-4 was easily exchanged in a time dependent manner during the recording of an ^{1}H NMR spectrum in deuterated pyridine supported an elimination–addition mechanism: Base catalysed elimination of glucose from 1 may give 11, which may then be transformed either to 4 by addition of methanol at the double bond or to 6 by cleavage of the lactone ring (see Fig. 2).

Similar reactions occurred during methylation of 1 with DMSO/NaH and methyl iodide according to ref. [10]. The permethylated product 9 was characterized by mass spectrometry. Under GC/MS conditions methylsulphenic acid is eliminated from 9 and the spectrum of 10 is recorded, which is in accord with our earlier observations on other iridoids [11].

EXPERIMENTAL

Plant material, V. brasiliensis was cultivated in the Botanical Garden, Freiburg. The aerial parts of the plant were collected in the flowering period. A voucher specimen (045-005) has been deposited at the herbarium of the Institut für Pharmazeutische Biologie, Freiburg.

Analytical methods. CC: silica gel (Merck, 40 μm), CH_2Cl_2–MeOH–H_2O (40:10:1, 70:30:3 and 15:10:1); Amberlite XAD-2 (Serva), H_2O–EtOH (9:1) and H_2O–MeOH (9:1), HPLC: μ-Bondapak C_{18} (18 × 250 mm), MeOH (25%), flow rate 8.0 ml/min. DCCC (ascending mode): *n*-PrOH–*n*-BuOH–H_2O (1:2:3). TLC: silica gel 60, CH_2Cl_2–MeOH–H_2O (70:30:3). Spray reagent: anisaldehyde–H_2SO_4–HOAc (0.5:1.0:50.0) followed by heating at 110° for 5–10 min. MS-direct inlet: Finnigan 4000, temp of source 140°, EIMS 30 eV. GC/MS: OV-17 (3% on Chromosorb W, AW-DMCS H.P. 80–100 mesh) column (1.2 m × 2 mm i.d.) injector 240°, column 180°, source 130° for penta-*O*-methylglucose, injector 250°, column 180°, source 140° for 3 and injector 250°, column 230°, source 140° for 9; He = 30 ml/min; Finnigan 4000, EIMS 30 eV. FDMS: Varian MAT 312, field desorption technique.

Table 1. ^{13}C NMR spectral data** of compounds **1**, **4** and **6** (63 MHz), brasoside (**5**), cistanin (**8**) and 6α-hydroxydihydrocornin (**7**) (20 MHz)

C	1* (D_2O)	4† ($CDCl_3$)	5‡ (D_2O)	8§ (d_5-pyr.)	6‖ ($CDCl_3$)	7¶ (D_2O)
1	57.58 *t* (151.8)††	54.77	96.71	101.4	69.85	101.3
3	98.02 *d* (169.2)	96.54	150.74	55.5	158.81	155.7
4	41.76 *d* (134.2)	40.90	104.13	25.0	0	106.8
5	34.69 *d* (147.4)	34.48	38.53	26.9	40.22	41.9
6	88.45 *d* (160.6)	86.29	84.54	37.0	78.43[a]	74.6
7	77.43 *d* (165.0)	77.44	41.70	79.2	79.23[a]	41.9
8	37.64 *d* (116.6)	37.26	32.24	88.7	38.51[b]	34.4
9	37.45 *d* (116.6)	36.52	45.57	47.1	37.43[b]	46.2
10	10.87 *q* (132.0)	10.68	17.40	71.5	13.19	21.3
11	178.99 *s*	0	175.13	—	0	170.8
1′	103.17 *d* (156.2)	—	99.51	—	—	99.9
2′	74.19 *d* (143.0)	—	73.45	—	—	73.5
3′	77.24 *d* (143.0)	—	76.33	—	—	76.6
4′	70.32 *d* (143.0)	—	70.37	—	—	70.4
5′	76.51 *d* (143.0)	—	77.11	—	—	77.1
6′	61.50 *t* (147.7)	—	61.50	—	—	61.5

Standards, **1** and **5** capillary d_4-TSPNa/D_2O as external standard; **4**, **6** and **8** TMS as internal standard.

*All carbons with a positive spin echo signal except C-1, C-11 and C-6′; assignment by selectively ^{1}H decoupled ^{13}C NMR spectroscopy.

†Additional signal for OMe-3 at δ55.10.

‡Additional signal for COOMe at δ51.71.

§From ref. [4].

‖From ref. [3].

¶From ref. [12], additional signal for COOMe at δ52.5.

[a,b]Interchangeable.

**Adjusted to δC-6′ = 61.50 ppm; chemical shift: 0 = signal with too low intensity; – = no signal observable.

††J (H_2) in parenthesis.

Enzymatic detection of β-D-glucose. Urine test strips (gluco-dip^R, Merck) were used to detect β-D-glucose by the glucose oxidase/peroxidase method (limit 1.1 mmol/l).

Isolation procedure. The lyophilized plant material (750 g) was extracted by refluxing for 30 min successively with 96% EtOH and with 80% EtOH. The combined extracts were evapd *in vacuo* and chromatographed on a Celite column. The lipophilic fraction obtained by elution with *n*-hexane was discarded and the hydrophilic fraction obtained by eluting with CH_2Cl_2–MeOH (1:1) was separated by CC on silica gel and XAD-2. Further purification by HPLC and subsequent DCCC yielded 178 mg vebraside (0.024% of the dry plant material).

Vebraside (**1**), Mp 131–133°. $[\alpha]_D^{23}$ + 80.7° (H_2O; *c* 0.195); UV $\lambda_{max}^{H_2O}$ (log ε)nm: 187.6 (3.25); IR ν_{max}^{KBr} cm^{-1}; 3600–3200, 2963, 2933, 2886, 2848, 1761, 1401, 1353, 1179, 1117, 1073, 1022, 984, 891, 840, 730; FDMS, *m/z*: 399 [M + Na]$^+$, 203 [glucose + Na]$^+$, 196 [M – glucose]$^+$; ^{13}C NMR: Table 1; ^{1}H NMR: Table 2.

Aglucone of vebraside (**2**, 4.0 mg). 10 mg **1** were treated with 1 mg β-glucosidase (Roth) in 4.0 ml H_2O and 0.2 ml citrate buffer (pH 4.9) for 2 days at 37°. The reaction mixture was extracted with EtOAc. The aq. phase contained glucose, which was identified by TLC and by a gluco-dip strip. The EtOAc phase contained **2**, which was purified by prep. TLC. IR ν_{max}^{KBr} cm^{-1}; 3600–3200, 2914, 2907, 2888, 2829, 1748, 1344, 1177, 1111, 1009; MS-direct inlet, *m/z* (rel. int.): 215 [M + 1]$^+$ (0.28), 214 [M]$^+$ (0.30), 197 (1.85), 179 (0.53), 168 (2.69), 159 (8.27), 150 (2.69), 140 (1.37), 139 (1.32), 123 (3.84), 122 (5.08), 113 (100), 85 (80.53), 84 (83.90), 55 (42.23).

TMSi derivative of **2** (**3**). Trimethylsylilation of compound **2** (0.17 mg) with 23 μl TMSI-S reagent (Serva) for 2 hr at room temp. gave a soln of **3**, which was directly injected for GC/MS analysis. GC/MS, *m/z* (rel. int.): 431 [M + TMS]$^+$ (0.12), 358 [M]$^+$ (0.27), 343 (3.82), 303 (0.59), 269 (0.52), 253 (8.51), 240 (2.47), 191 (42.07), 185 (30.68), 179 (2.39), 157 (45.84), 150 (1.92), 129 (35.45), 123 (1.97), 122 (3.09), 103 (16.62), 75 (99.07), 73 (100), 55 (29.96).

Methanolysis. To 20 mg **1** dissolved in 0.4 ml MeOH, 0.6 ml 0.05 M Na-methylate was added. After stirring the mixture for 3 hr at room temp., the reaction was stopped by neutralization with 0.1 M HCl. After evaporation of the solvent and addition of H_2O the methyl derivatives **4** and **6** were extracted with $CHCl_3$ purified by prep. TLC (silica gel, CH_2Cl_2–MeOH–H_2O, 40:10:1) and identified by IR and MS; β-D-glucose was identified enzymatically in the aq. phase.

Compound **4** (3.6 mg). IR ν_{max}^{film} cm^{-1}: 3600–3200, 2917, 2869, 2850, 1747, 1342, 1170, 1158, 1121, 1054, 1015; MS-direct inlet, *m/z* (rel. int.): 228 [M]$^+$ (2.20), 213 (195), 211 (5.26), 197 (2179), 179 (17.81), 173 (51.52), 168 (13.89), 150 (8.78), 140 (7.07), 139 (7.85), 123 (54.49), 122 (17.52), 113 (100), 85 (96.55), 55 (61.14).

Compound **6** (4.2 mg). IR ν_{max}^{film} cm^{-1}: 3600–3200, 2912, 2888, 1653, 1618, 1429, 1378, 1256, 1181, 1087, 1044; MS-direct inlet,

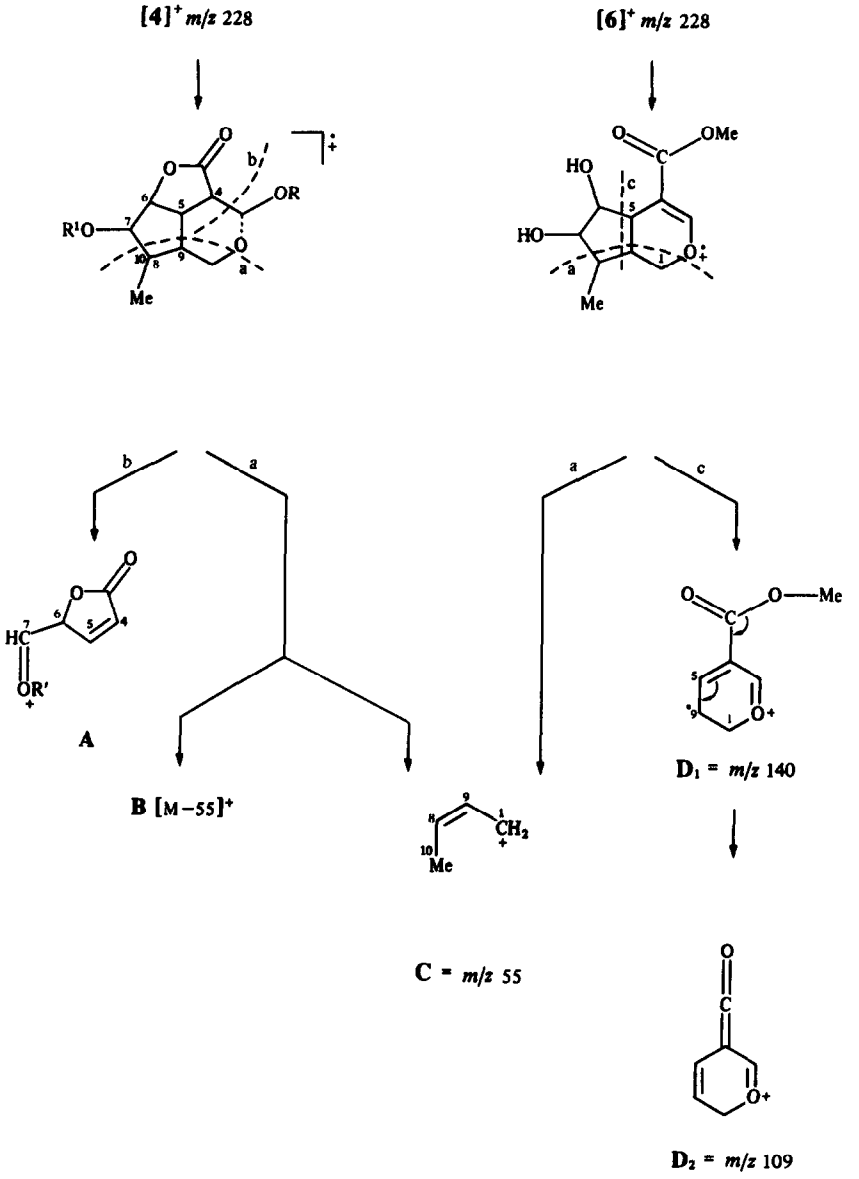

Fig. 1.

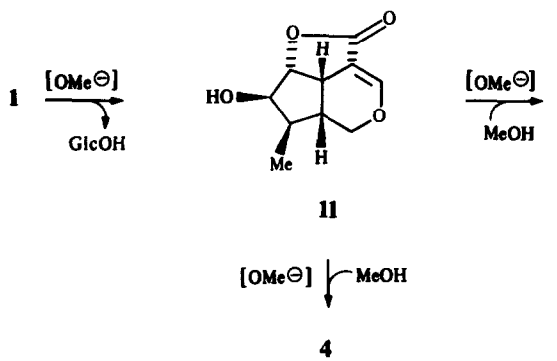

Fig. 2.

m/z (rel. int.): 229 $[M+1]^+$ (0.51), 228 (2.23), 210 (10.53), 196 (51.37), 195 (6.01), 178 (27.36), 168 (9.89), 167 (11.19), 163 (8.05), 151 (7.80), 150 (8.00), 140 (35.24), 125 (25.69), 121 (16.47), 109 (100), 81 (59.91), 55 (91.67).

Methylation. 18 mg **1** were methylated using $NaCH_2SOCH_3$ and CH_3I in DMSO [9–11]. Pure permethylether were obtained by prp. TLC (silica gel, CH_2Cl_2–MeOH–H_2O, 90:10:1) and identified by IR and MS.

Compound **9** (8.3 mg). Obtained by methylation of **1**. IR ν_{max}^{film} cm^{-1}: 2892, 2844, 1748, 1174, 1095, 1047, 1039, 945, 800; MS-direct inlet, *m/z* (rel. int.): 393 $[M-1]^+$ (0.14), 375 (0.85), 363 (0.29), 361 (0.58), 349 (2.53), 331 (32.21), 317 (23.41), 299 (18.73), 285 (4.32), 2.83 (3.61), 267 (15.40), 253 (13.45), 235 (19.51), 221 (18.48), 207 (14.62), 193 (13.35), 189 (29.27), 175 (46.47), 155 (58.97), 123 (100), 111 (100), 105 (92.18), GC/MS giving **10**, *m/z* (rel. int.): 330 $[M]^+$ (0.44), 315 (0.44), 299 (1.21), 285 (0.55), 281

Table 2. ^{1}H NMR spectral data* of compounds 1, 4, 5 and 6 (250 MHz)

	1§ (D_2O, TSPNa/$d_5$pyr., TMS)	4‖ ($CDCl_3$, TMS)	5‡ (D_2O, TSPNa)	6¶ ($CDCl_3$, TMS)
H-1α	3.68 *d*(*br*)/3.69 *d*(*br*) $J_{1\alpha,1\beta}$ = 13.2/13.0	3.42 *d*(*br*) $J_{1\alpha,1\beta}$ = 12.5 $J_{1\alpha,9}$ = 0.5	5.73 *s*(*br*)	3.51 *dd*(*br*) $J_{1\alpha,1\beta}$ = 11.0 $J_{1\alpha,9}$ = 10.5 $J_{1\alpha,3}$ = 1.0
H-1β	4.39 *dd* /4.83 *dd* $J_{1\beta,1\alpha}$ = 13.2/13.0 $J_{1\beta,9}$ = 3.5/3.5	3.95 *dd* $J_{1\beta,1\alpha}$ = 12.5 $J_{1\beta,9}$ = 3.5		4.17 *dd*(*br*) $J_{1\beta,1\alpha}$ = 11.0 $J_{1\beta,9}$ = 5.5 $J_{1\beta,3}$ < 0.5
H-3	5.47 *s*(*br*)/6.22 *s*(*br*) $J_{3,4}$ < 1/ < 1	4.94 *s*(*br*) $J_{3,4}$ = 0.2 $J_{3,5}$ = 0.5	7.3 *d* $J_{3,5}$ = 2.0	7.78 *dd*(*br*) $J_{3,1\beta}$ < 0.5 $J_{3,1\alpha}$ = 1.0 $J_{3,5}$ = 1.5
H-4	3.22 *d*(*br*)/3.27 *d*(*br*) $J_{4,3}$ < 1/ < 1 $J_{4,5}$ = 10.5/10.5	2.66 *dd*(*br*) $J_{4,3}$ = 0.2 $J_{4,5}$ = 11.0 $J_{4,9}$ = 0.8	—	—
H-5	3.5 *m*/3.5 *m* $J_{5,4}$ = 10.5/10.5 $J_{5,6}$ = 7.0/7.0 $J_{5,9}$ = 0/9.5	3.24 *m* $J_{5,4}$ = 11.0 $J_{5,6}$ = 6.5 $J_{5,9}$ = 9.0 $J_{5,3}$ = 0.5	3.4 *m*	3.37 *ddd* $J_{5,3}$ = 1.5 $J_{5,6}$ = 4.0 $J_{5,9}$ = 8.5
H-6	4.96 *d*(*br*)/4.96 *d*(*br*) $J_{6,5}$ = 7.0/7.0 $J_{6,7}$ < 1/ < 1	4.67 *d*(*br*) $J_{6,5}$ = 6.5 $J_{6,7}$ = 0.3	5.07 *dd* $J_{6',5}$ = 7.5 $J_{6,7A}$ = 8.3	4.35 *d*(*br*) $J_{6,5}$ = 4.0 $J_{6,7}$ = 2.0
H-7	4.20 *d*(*br*)/4.31 *d*(*br*) $J_{7,6}$ < 1/ < 1 $J_{7,8}$ = 3.5/3.5	4.09 *m* $J_{7,6}$ = 0.3 $J_{7,8}$ = 3.5 $J_{7,9}$ = 0.2	A: 1.59 *ddd* $J_{7A,7B}$ = 14.2 $J_{7A,8}$ = 10.5 $J_{7A,6}$ = 8.3 B: 1.95 *dd* $J_{7B,7A}$ = 14.2 $J_{7B,8}$ = 7.5	3.97 *dd*(*br*) $J_{7,6}$ = 2.0 $J_{7,8}$ = 4.0
H-8	1.94 *m*/2.16 *ddq* $J_{8,7}$ = 3.5/3.5 $J_{8,9}$ = —/12.0 $J_{8,10}$ = 5.5/5.5	2.01 *m* $J_{8,7}$ = 3.5 $J_{8,9}$ = 12.0 $J_{8,10}$ = 7.0	1.8 *m*	1.89 *m* $J_{8,7}$ = 4.0 $J_{8,10}$ = 7.0
H-9	1.94 *m*/1.97 *ddd* $J_{9,1\beta}$ = 3.5/3.5 $J_{9,5}$ = †/9.8 $J_{9,8}$ = †/12.0	1.76 *ddd*(*br*) $J_{9,1\beta}$ = 3.5 $J_{9,5}$ = 9.0 $J_{9,8}$ = 13.0 $J_{9,1\alpha}$ = 0.5 $J_{9,4}$ = 0.5	2.06 *dd* $J_{9,5}$ = 6.8 $J_{9,8}$ = 10.5	1.85 *m* $J_{9,1\beta}$ = 5.5 $J_{9,5}$ = 8.5 $J_{9,1\alpha}$ = 10.5
H_3-10	1.07 *d*/1.08 *d* $J_{10,8}$ = 5.5/5.5	0.99 *d* $J_{10,8}$ = 7.0	0.9 *d* $J_{10,8}$ = 6.0	1.08 *d*(*br*) $J_{10,8}$ = 7.0

*δ-Values in ppm related in standard; J-values in Hz.

†Chemical shift/coupling constant not analysed.

— No signal observable.

‡All data from ref. [3].

§β-D-Glucosyl moiety of 1 in D_2O: 4.74 (H-1', *d*, $J_{1',2'}$ = 7.5 Hz), 3.37 (H-2', *dd*, $J_{2',3'}$ = 8.5 Hz), 3.57 (H-3', *dd*, $J_{3',4'}$ = 8.5 Hz), 3.45 (H-4', *dd*, $J_{4',5'}$ = 8.5 Hz), 3.52 (H-5', *m*), 3.78 (H-6'A, *dd*, $J_{6'A,5'}$ = 5.2 Hz, $J_{6'A,6'B}$ = 12.0 Hz), 3.90 (H-6'B, *dd*, $J_{6'B,5'}$ = 2.8 Hz).

‖Additional signal for OMe-e at 3.35 ppm (*s*) and for OH-7 at 1.56 ppm (*br*, *s*).

¶Additional signal for COOMe at 3.69 ppm (*s*).

(0.55), 269 (0.66), 267 (0.44), 253 (2.10), 235 (0.66), 221 (6.20), 207 (3.10), 203 (0.99), 193 (3.22), 189 (1.66), 181 (1.66), 175 (25.13), 161 (6.86), 145 (17.27), 133 (12.07), 123 (24.91), 111 (15.83), 105 (21.37), 91 (24.03), 85 (41.52), 45 (100).

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Table 3. Results of homonuclear spin–spin decoupling experiments and NOEs in the ^{1}H NMR spectrum (250 MHz, TMS, d_5-pyridine) of compound 1

Irradiation at	Original signal	Affected proton (new signal)	NOE*
H-1α	*d(br)*	†	H-1β, H_3-10
H-1β	*dd*	H-1α (*br, s*), H-9 (*dd*)	H-1α, H-9
H-3	*s(br)*	†	H-4, H-1′
H-4	*d(br)*	H-5 (*dd*) H-3 (*s*)	H-3, H-5
H-5	*m*	H-9 (*dd*), H-6 (*s*), H-4 (*s*)	H-4, H-9, H-6
H-6	*d(br)*	H-5 (*m*), H-7 (*d*)	H-5, H-7
H-7	*d(br)*	H-6 (*d*), H-8 (*d*)	H-6, H-8
H-8	*ddq*	H-7 (*s*), H-9 (*m*), H_3-10 (*s*)	H-7, H_3-10
H-9	*ddd*	H-8 (*m*), H-5 (*dd*), H-1β (*d*)	H-5, H-1β, H_3-10
H_3-10	*d*	H-8 (*dd*)	H-8, H-9. H-1α

*Positive NOE on the H-atoms listed.
†No decoupling experiments carried out.

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