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6-Hydroxyluteolin-7-*O*-(1′′-α-rhamnoside) from *Vriesea sanguinolenta* Cogn. and Marchal (Bromeliaceae)

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Abstract

The isolation of 6-hydroxyluteolin-7-*O*-(1′′-α-rhamnoside) from the Central American epiphyte *Vriesea sanguinolenta* Cogn. and Marchal (Bromeliaceae) is described here. Its stereostructure was established by spectroscopic methods and an X-ray structure analysis of its hepta-*O*-acetyl derivative. This flavonoid glycoside had previously been reported from some *Teucrium* species (Labiatae), yet without sufficient physical data and spectroscopic evidence. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Vriesea sanguinolenta*; Bromeliaceae; Structural elucidation; X-ray crystallography; 6-Hydroxyluteolin-7-*O*-(1′′-α-rhamnoside); 5,6,3′,4′-Tetra-*O*-acetyl-6-hydroxyluteolin-7-*O*-(1′′-α-2′′,3′′,4′′-tri-*O*-acetylrhamnoside); Antimalarial activity

1. Introduction

The epiphytic bromeliad *Vriesea sanguinolenta* Cogn. and Marchal is distributed throughout Central and South America, including the Caribbean (Croat, 1978). It has been the subject of recent examinations of the spatial distribution and the substrate preferences of co-occurring epiphytes (Zotz, 1997). Ongoing studies on the protection of epiphytes from damage by UV irradiation and herbivory prompted us to investigate the secondary metabolites of *V. sanguinolenta*. In the course of this work, we have isolated a flavonoid glycoside that had not yet been reported from bromeliads nor even from any monocotyledonous plants. The isolation and structural elucidation of this metabolite are described in this paper, and its chemotaxonomical significance is discussed.

2. Results and discussion

The methanolic extract of the dried and powdered leaves was suspended in water and centrifuged. After subjecting the supernatant to CC on Sephadex LH-20, an amorphous yellow powder was obtained. The ^{1}H-NMR signals of the isolated compound between 3.1 and 4.0, at 5.52 ppm (see Fig. 1(a)), and between 6.6 and 7.5 ppm, in connection with a corresponding pattern in the ^{13}C-NMR spectrum indicated typical features (Agrawal, 1992; Markham, 1982) of a glycosylated flavonoid. Due to the fact that the ^{13}C-NMR spectrum showed 21 carbon atoms, the molecular formula $C_{15}H_{10}O_7^+$ for $m/z = 302$ obtained from HREIMS was likely to correspond to a fragment ion — presumably the aglycone. The CIMS yielded an

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apparent $[M]^+$ peak at $m/z = 448$. From the mass difference of $m/z = 146$ between the $[M]^+$ peak and the aglycone it was deduced that the sugar moiety was a deoxyhexose. A doublet at 1.14 ppm in the ^{1}H-NMR spectrum (see Fig. 1(a)), the H,H-COSY interactions, the ^{13}C shifts deduced from 2D NMR experiments, and the ^{1}H-NMR coupling constants (Bock & Thøgersen, 1982; Bock & Pedersen, 1983) made an α-rhamnose-*O*-glycoside likely.

For the generation of yet another spectroscopic data set and for the determination of the number of hydroxy groups, the compound was peracetylated. The DCIMS of the derivative yielded an $[M+NH_4]^+$ peak with $m/z = 760$, corresponding to $M_r = 742$ and thus indicating the presence of seven acetyl groups. This was confirmed by the appearance of seven signals between 2.0 and 2.5 ppm in the ^{1}H-NMR spectrum, which integrated to three hydrogen atoms each and which therefore belonged to the acetyl groups, demonstrating the same number of hydroxy groups to be present in the underivatized compound. Because three of the seven OH groups were attributable to the rhamnose moiety, the remaining four had to be attached to the flavonoid aglycone.

From the coupling pattern in the ^{1}H-NMR spectrum and the Rotating Frame Overhauser Enhancement Spectroscopy (ROESY) interactions (see Fig. 1(a)), two of the five aromatic protons were assigned to the chromone part of the aglycone and the remaining three were attributed to the phenyl moiety. The latter revealed a 1′,3′,4′-substitution pattern, in which one substituent corresponded to the chromone moiety, while the remaining two should be represented by hydroxy functionalities. The attachment positions between the phenyl ring and the chromone portion were indicated as C-2 and C-1′, respectively, by the corresponding Heteronuclear Multiple Bond Correlation (HMBC) and ROESY interactions (see Fig. 1(b) and 1(a)). According to this, C-1′ showed an HMBC-coupling only to the singlet of 3-H (6.71 ppm), but not to 8-H (6.94 ppm) (see Fig. 1(b)). The attribution of these two singular proton signals was, therefore unambiguous. Furthermore, only the signal at 6.94 ppm (8-H) displayed HMBC interactions with C-6 and C-7, the latter of which in turn interacted with H-1′′ of the sugar moiety. The ROESY correlation between 8-H (6.94 ppm), 1′′-H and 5′′-H confirmed the resulting vicinity between C-8 and the *O*-glycosidically bound hexose. Moreover, the HMBC interaction between 1′′-H and C-7 proved the sugar to be linked via the oxygen at C-7. Any other substitution pattern within the chromone ring was excluded by the HMBC relationships furtherxs observed. This chain of arguments was analogously applied to the heptaacetyl derivative **2**, and this led in conclusion to the structure of 6-hydro-

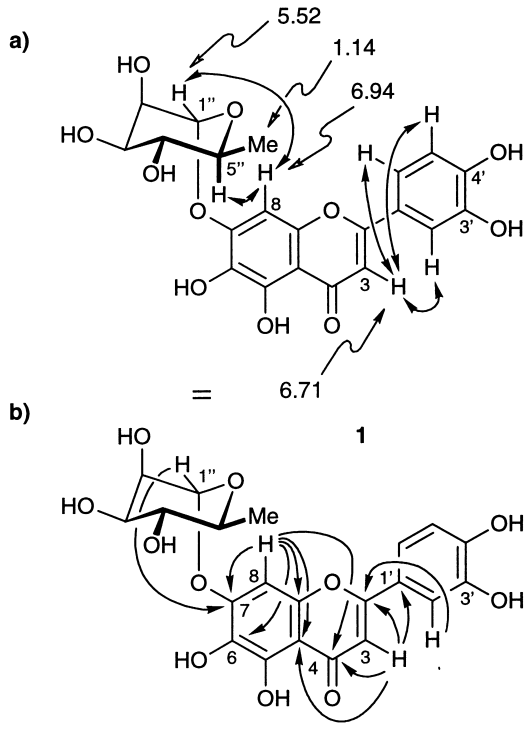

Fig. 1. Structure of the glycoside **1** as deduced from chemical shifts and selected ROESY (a) and HMBC (b) interactions.

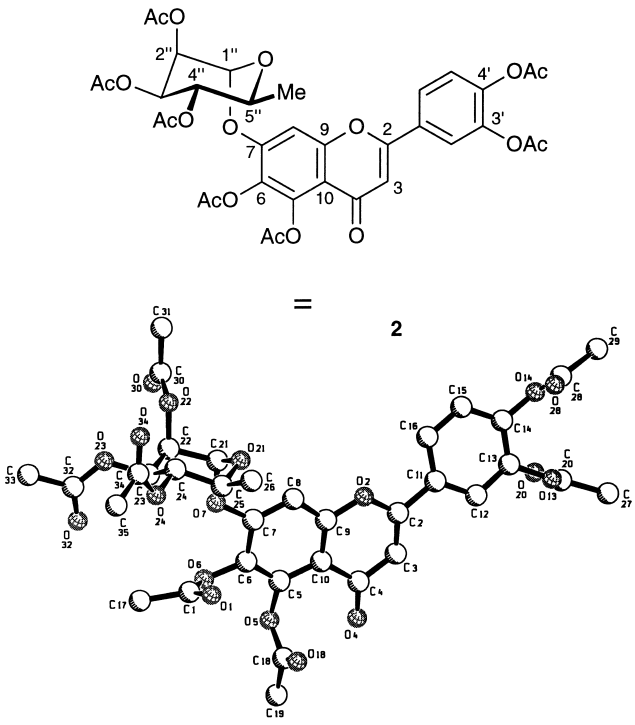

Fig. 2. Stereostructure of **2** in the crystal; hydrogen atoms have been omitted for reasons of clarity.

xyluteolin-7-*O*-(1′′-α-rhamnoside) (**1**) for the isolated flavonoid glycoside (Fig. 1).

From the peracetyl derivative, which thus had the structure **2**, crystals were obtained, of which an X-ray diffraction analysis (see Fig. 2) fully confirmed the structure deduced from the spectra, in all details.

Both compounds, **1** and **2**, showed moderate antimalarial activities in vitro against *Plasmodium falciparum*, using chloroquine resistant (IC_{50} [K1-strain] = 2.13 μM for **1** and 0.52 μM for **2**) and susceptible (IC_{50} [NF54-strain] = 3.32 μM for **1** and 0.63 μM for **2**) strains. For an example of another antiplasmodial flavonoid, quercetin, see Khalid, Farouk, Geary and Jensen (1986).

The 6-hydroxyluteolin glycoside **1** had previously been detected in several *Teucrium* species (Labiatae) by TLC (Harborne, Tomas-Barberan, Williams & Gil, 1986). In those investigations, the structural elucidation was carried out by acid hydrolysis and identification of the aglycone and the sugar just by TLC comparison, with no spectroscopic or physical data being given except for an R_f value. The compound is, therefore, fully characterised and structurally established in this paper for the first time.

While the flavonoid glycoside **1** had so far only been detected in some *Teucrium* species (Harborne et al., 1986), the aglycone was found in a free form or as differently glycosidized derivatives in Bromeliaceae and various families of 'asterids' (Williams, 1978; Ranganathan, Nagarajan, Mabry & Yong-Long, 1980; Schaufelberger & Hostettmann, 1984; Klimek, 1988; Harborne, 1994; Harborne & Williams, 1979). The presence of **1** in *V. sanguinolenta* underlines the parallel between the monocot Bromeliaceae and the dicot 'asterids', since both of the two lineages can apparently hydroxylate the flavonoid skeleton at C_6. This ability has been assumed to represent a phylogenetically young trait (Harborne & Williams, 1979). The chemoecological significance of **1** shall be investigated in future field studies.

3. Experimental

3.1. General

Mps uncorr. Optical rotation: 10 cm cell, $CHCl_3$. IR: KBr. ^{1}H-NMR (600 MHz, Bruker) and ^{13}C-NMR (150 MHz, Bruker) were recorded in $CDCl_3$ (solvent as internal standard, δ 7.26 and δ 77.01 respectively), DMSO-d_6 (solvent as internal standard, δ 2.50 and δ39.43, respectively). Proton detected, heteronuclear correlations were measured using Heteronuclear Multiple Quantum Correlation (HMQC, optimized for $^1J_{HC} = 150$ Hz) and HMBC (optimized for $^nJ_{HC} = 7$ Hz). EIMS: 70 eV. CIMS: NH_3 as the reagent gas, electron energy 70 eV, source pressure held at 0.3 mbar and temperature at 130–140°C. DCIMS: NH_3 as the reagent gas, electron energy 70 eV, source pressure held at 0.3 mbar and temperature at 130–140°C. CC: Sephadex LH-20 (Pharmacia), silica gel (0.032–0.063 mm, Merck). TLC: precoated silica gel 60 F_{254} plates (Merck), spots were detected under UV light. The X-ray data were obtained at room temp. on a Siemens P4 diffractometer using graphite monochromatized Mo*K*α radiation.

3.2. Plant material

Green leaves from mature individuals of *V. sanguinolenta* Cogn. and Marchal were collected in Barro Colorado Nature Monument (Aojeta Bay), Panama (G.Z.). Voucher specimen have been deposited at the Herbarium Bringmann, Würzburg, Germany (no. 41), and at the Herbarium of Barro Colorado Island, Panama.

3.3. 6-Hydroxyluteolin-7-O-(1′′-α-rhamnoside) (*1*)

Lyophilized leaf chips (200 g dry weight) of *V. sanguinolenta* were ground to give a powder, and extracted with MeOH (2.4 l). After evaporation of the solution under reduced pressure, the dark-green residue (50 ml) was suspended in water (50 ml) and centrifuged (10,000 g, 4°C, 10 min). The supernatant was lyophilized, redissolved in MeOH, and subjected to CC on Sephadex LH-20 with MeOH as the eluent. Of the 10-ml fractions collected, fractions 7–9, which contained **1** in a pure form, were pooled. Evaporation of the solution under reduced pressure gave an amorphous yellow powder (50.0 mg).

$[\alpha]_D$: Not measured due to the insolubility of **1** in MeOH, H_2O, and halogenated solvents. IR ν_{max} cm^{-1}: 3600–3000 (O–H), 1650 (C═O), 1590 (C═C), 1480, 1460, 1360, 1350, 1260, 1080. ^{1}H-NMR (600 MHz, DMSO-d6): δ 1.14 (3H, *d*, $J = 6.2$ Hz, CH_3), 3.17 (1H, *s*, OH), 3.32 (1H, *dd*, $J = 9.4$ Hz, $J = 9.4$ Hz, 4′′-H), 3.52 (1H, *dd*, $J = 9.3$ Hz, $J = 6.2$ Hz, 5′′-H), 3.81 (1H, *dd*, $J = 9.4$ Hz, $J = 3.4$ Hz, 3′′-H), 3.93 (1H, *br.*, 2′′-H), 4.50–5.40 (3H, *br.*, OH), 5.52 (1H, *d*, $J = 1.3$ Hz, 1′′-H), 6.71 (1H, *s*, 3-H), 6.89 (1H, *d*, $J = 8.0$ Hz, 5′-H), 6.94 (1H, *s*, 8-H), 7.44 (1H, s, 2′-H, overlapped with 6′-H), 7.45 (1H, m_c, 6′-H, overlapped with 2′-H), 8.30–10.30 (3H, *br.*, OH), 12.75 (1H, *br.*, OH). ^{13}C-NMR (150 MHz, DMSO-d_6): δ 17.85 (C-6′′), 69.80 (C-2′′), 69.88 (C-5′′), 70.00 (C-3′′), 71.65 (C-4′′), 94.23 (C-8), 99.16 (C-1′′), 102.32 (C-3), 105.57 (C-10), 113.32 (C-2′), 115.93 (C-5′), 118.94 (C-6′), 121.49 (C-1′), 130.79 (C-6), 145.69 (C-3′), 146.85 (C-5), 148.86 (C-9), 149.67 (C-4′), 150.58 (C-7), 164.10 (C-2), 182.12 (C-4). The ^{13}C attributions were achieved by HMQC and HMBC experiments. EIMS *m*/*z* (rel. int.): 302

$[C_{15}H_{10}O_7]^+$ (100), 286 $[302\text{-}H_2O]^+$ (12), 168 (57), 69 (36), 43 (31). CIMS (NH_3) m/z (rel. int.): 448 $[M]^+$ (0.2), 180 (30), 163 (52), 146 (100), 128 (26). HREIMS: $C_{15}H_{10}O_7$. Found 302.043, calc. 302.043.

3.4. Acetylation of **1**

A mixture of 20.0 mg (45 μmol) of **1** in 0.5 ml of Ac_2O and 0.5 ml of pyridine was stirred 20 h at room temperature. The solution was concentrated to dryness in vacuo and purified by chromatography on a silica gel column, with CH_2Cl_2–MeOH (99:1 → 94:6) as the eluent. The product, 5,6,3′,4′-tetra-*O*-acetyl-6-hydroxyluteolin-7-*O*-(1′′-α-2′′,3′′,4′′-tri-O-acetylrhamnoside) (**2**), was obtained as colorless needles (28.1 mg, 37 μmol, 83%) from EtOH–CH_2Cl_2. Mp 266°C. $[\alpha]_D^{25}$ −60.1° ($CHCl_3$, *c* 0.32). IR ν_{max} cm^{-1}: 1800–1700 (C═O), 1630 (C═C), 1550, 1250–1150. ^1H NMR (600 MHz, $CDCl_3$): δ 1.25 (3H, *d*, $J = 6.2$ Hz, CH_3), 2.03 (3H, *s*, CO_2CH_3 at C-3′′), 2.08 (3H, *s*, CO_2CH_3 at C-6), 2.20 (3H, *s*, CO_2CH_3 at C-2′′), 2.32 (3H, *s*, CO_2CH_3 at C-3′ or C-4′), 2.35 (3H, *s*, CO_2CH_3 at C-3′ or C-4′), 2.45 (3H, *s*, CO_2CH_3 at C-7 or C-4′′), 2.46 (3H, *s*, CO_2CH_3 at C-7 or C-4′′), 3.92 (1H, *dd*, $J = 9.7$ Hz, $J = 6.2$ Hz, 5′′-H), 5.20 (1H, *dd*, $J = 10.1$ Hz, $J = 10.0$ Hz, 4′′-H), 5.33 (1H, *dd*, $J = 10.3$ Hz, $J = 3.3$ Hz, 3′′-H), 5.41 (1H, *br.*, 2′′-H), 5.60 (1H, *d*, $J = 1.7$ Hz, 1′′-H), 6.58 (1H, *s*, 3-H), 7.24 (1H, *s*, 8-H), 7.35 (1H, *d*, $J = 8.5$ Hz, 5′-H), 7.68 (1H, *d*, $J = 2.1$ Hz, 2′-H), 7.71 (1H, *dd*, $J = 8.5$ Hz, $J = 2.1$ Hz, 6′-H). ^{13}C-NMR (151 MHz, $CDCl_3$): δ 17.47 (C-6′′), 20.01 ($COCH_3$), 20.60 ($COCH_3$), 20.64 ($COCH_3$), 20.78 ($COCH_3$), 20.82 ($COCH_3$), 68.47 (C-3′′ or C-5′′), 68.50 (C-3′′ or C-5′′), 68.99 (C-2′′), 69.82 (C-4′′), 95.96 (C-1′′), 101.16 (C-8), 108.58 (C-3), 112.68 (C-10), 121.49 (C-2′), 124.29 (C-5′), 124.43 (C-6′), 129.68 (C-1′), 130.96 (C-6), 142.01 (C-5), 142.63 (C-3′), 144.77 (C-4′), 151.81 (C-7), 155.06 (C-9), 160.50 (C-2), 167.74 ($COCH_3$), 167.99 ($COCH_3$), 168.18 ($COCH_3$), 168.66 ($COCH_3$), 169.76 ($COCH_3$), 169.96 ($COCH_3$), 175.95 (C-4). The ^{13}C attributions were achieved by HMQC and HMBC experiments. EI-measurements showed decomposition products, no $[M]^+$ peak. CIMS (NH_3) m/z (rel. int.): 760 $[M + NH_4]^+$ (100), 743 $[M + H]^+$ (6), 718 $[M\text{-}C_2H_3O + H]^+$ (10), 408 (14), 290 $[C_{12}H_{18}O_8]^+$ (7), 273 $[C_{12}H_{17}O_7]^+$ (7), 137 (34).

3.5. X-Ray structure analysis of compound **2**

Crystals suited for an X-ray structure analysis were obtained from EtOH–CH_2Cl_2. Crystal dimensions, 0.25 × 0.3 × 0.75 mm, $C_{35}H_{34}O_{18}$, $M_r = 742.64$, hexagonal, a = 15.715(1), c = 26.289(2) Å; $V = 5623(1) \times 10^6 Å^3$, space group $P6_1$, $Z = 6$, $D_c = 1.316$ g cm^{-3}, μ(Mo*K*α) = 0.11 mm^{-1}, F(000) = 2328. The final refinement converged to $R = 0.113$ and $R_W = 0.071$. Atomic coordinates, bond lengths and angles, and thermal parameters may be obtained from the Cambridge Crystallographic Data Centre on quoting the depository number CCDC 133321, Table 1.

Table 1
Atomic parameters (×10^4) and equivalent isotropic displacement parameters ($pm^2 \times 10^{-1}$) of nonhydrogen atoms for compound **2**

Atom	*x*	*y*	*z*	U_{eq}
O(1)	−4748(3)	7119(4)	505	94(3)
C(1)	−4737(5)	7196(4)	53(3)	71(3)
O(2)	−507(2)	8097(2)	583(2)	53(2)
C(2)	315(4)	8995(4)	623(3)	53(3)
C(3)	307(4)	9841(4)	513(3)	59(3)
C(4)	−553(4)	9846(4)	354(3)	59(3)
O(4)	−560(3)	10598(3)	267(3)	85(2)
C(5)	−2313(4)	8673(3)	77(3)	54(3)
O(5)	−2423(3)	9439(2)	−113(2)	66(2)
C(6)	−3088(4)	7753(4)	20(3)	53(3)
O(6)	−3934(3)	7582(3)	−228(2)	64(2)
C(7)	−2990(4)	6939(4)	161(3)	51(3)
O(7)	−3819(2)	6043(2)	62(3)	62(2)
C(8)	−2134(4)	7061(3)	366(3)	50(2)
C(9)	−1356(4)	8023(3)	407(3)	49(3)
C(10)	−1425(4)	8843(3)	287(3)	49(3)
C(11)	1200(4)	8931(4)	773(3)	52(3)
C(12)	2025(4)	9747(4)	955(3)	60(3)
C(13)	2851(4)	9673(4)	1055(3)	63(3)
O(13)	3678(3)	10486(3)	1275(3)	83(2)
C(14)	2881(4)	8834(4)	969(3)	58(3)
O(14)	3767(3)	8863(3)	1023(3)	83(2)
C(15)	2060(5)	8032(4)	784(3)	70(3)
C(16)	1201(4)	8064(4)	689(3)	55(3)
C(17)	−5650(4)	6822(4)	−277(3)	91(4)
C(18)	−2571(5)	10000(5)	214(4)	72(3)
O(18)	−2770(4)	9805(4)	640(3)	106(3)
C(19)	−2497(5)	10868(4)	−44(4)	112(4)
C(20)	4363(5)	11090(5)	941(4)	89(4)
O(20)	4267(4)	10997(4)	489(3)	127(3)
C(21)	−3828(4)	5154(3)	169(3)	53(3)
O(21)	−3881(2)	4959(2)	683(2)	53(2)
C(22)	−4700(4)	4383(4)	−138(3)	67(3)
O(22)	−4630(3)	3493(3)	−110(3)	76(2)
C(23)	−5640(4)	4170(4)	122(3)	63(3)
O(23)	−6470(3)	3383(3)	−127(3)	98(2)
C(24)	−5631(3)	3915(4)	660(3)	58(3)
O(24)	−6525(3)	3742(3)	897(3)	80(2)
C(25)	−4778(4)	4773(4)	931(3)	55(3)
C(26)	−4705(5)	4564(5)	1482(3)	85(4)
C(27)	5233(5)	11890(5)	1212(4)	118(4)
C(28)	3923(5)	8532(6)	1453(4)	95(4)
O(28)	3321(4)	8188(6)	1768(3)	181(5)
C(29)	4935(5)	8687(6)	1467(4)	137(6)
C(30)	−4251(5)	3274(5)	−508(4)	94(4)
O(30)	−3987(5)	3763(4)	−877(3)	169(5)
C(31)	−4234(6)	2356(5)	−422(4)	142(5)
C(32)	−7060(8)	3558(7)	−361(6)	182(7)
O(32)	−6930(7)	4349(5)	−425(5)	328(8)
C(33)	−8002(7)	2701(6)	−533(5)	334(13)
C(34)	−7165(5)	2813(5)	1055(4)	93(4)
O(34)	−7006(4)	2179(3)	1038(3)	146(4)
C(35)	−8080(4)	2750(6)	1245(4)	133(5)

3.6. Biological experiments

Antiplasmodial activities were tested following the procedures published previously (Bringmann, Wenzel, Kelly, Boyd, Gulakowski & Kaminsky, 1999).

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