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6-*O*-β-D-XYLOPYRANOSYLAUCUBIN FROM *VERBASCUM SINUATUM**

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Key Word Index—*Verbascum sinuatum* L.; Scrophulariaceae; iridoid diglycosides; D-xylose.

Abstract—*Verbascum sinuatum* contains, in addition to aucubin and harpagide, four new highly polar iridoid glycosides one of which has been identified as 6-*O*-β-D-xylopyranosylaucubin on the basis of spectral data and chemical modifications.

INTRODUCTION

During our research on iridoid glycosides of Scrophulariaceae we examined the ethanolic extract of *Verbascum sinuatum* (aereal part harvested at summer time). We found that the plant contains, besides aucubin (**1**, R_f 0.29) as major component [2], harpagide [3] (R_f 0.26) and four highly polar compounds (R_f 0.19, 0.13, 0.05 and 0.04) all giving an aucubin-type chromatic reaction.

We now report the elucidation of the structure and configuration of the compound with R_f 0.13 (**2**), which was found to contain a D-xylose unit, relatively rare in iridoid diglycosides.†

RESULTS AND DISCUSSION

Compound **2** was a crystalline compound with molecular formula $C_{20}H_{30}O_{13}$, $[\alpha]_D$ −85° and mp 192–193. Its UV (204 nm, log $\varepsilon = 3.6$) and IR (1650 cm^{-1}) absorptions were in accordance with the presence of a non-conjugated iridoid enol–ether system. The very low R_f value was similar to those of other diglycosyliridoids, e.g. 10-*O*-β-glucosyl aucubin (**3**, R_f 0.07) [6] and ulmoside (**4**, aucubigenin 1-*O*-β-isomaltoside, R_f 0.11) [7]. The acid hydrolysis of **2**, carried out in refluxing N H_2SO_4, afforded two different monoses (1 mol each), with R_f 0.16 (**5**) and 0.28 (**6**), together with black products arising from aglycone decomposition. The $[\alpha]_D$ and R_f values and the ^{1}H NMR spectra of the monoses allowed **5** to be identified as D-glucose and **6** as D-xylose, as confirmed by further comparison with authentic samples.

As milder hydrolysis conditions did not reveal any disaccharide production, the presence in **2** of **5** and **6** in a glycobiose unit was considered very improbable. (The glycosidic linkage of iridoids is far more easily hydrolysed than the interglycosidic one.)

The ^{1}H NMR spectrum of **2** besides confirming an iridoid structure showed for the aglycone protons a signal pattern very similar to that of aucubin (**1**) except for the small downfield shift (0.09 ppm) observed for H-7. In **2** the H-5 and H-9 resonances, partly masked by the upfield part of the glucosidic signals, were not easily distinguishable at 90 MHz although spin decoupling experiments allowed the establishment of their absorption range (δ 3.2–2.9).

Regarding the anomeric protons of **2**, two signals appeared as doublets, one at δ 4.80‡ ($J_{1',2'} = 7.5$ Hz) assigned to the H-1′ of the β-D-glucose (δ 4.78 in **1**) and the other at 4.58 ($J_{1'',2''} = 7.4$ Hz) to the β-D-xylose H-1″ proton. The large coupling constants prove the β-configuration of both anomeric centres [8]. As expected the region of the remaining glycosidic protons (4.2–3.1) had a larger integral value than in **1**.

In order to demonstrate the aucubigenin structure for the aglycone of **2** we carried out the enzymatic hydrolysis of **2** with snail (*Helix pomatia*) hepatopancreatic juice. The aglycone (obtained in low yield) was readily acetylated in mild conditions giving the triacetate (peracetate) **7**, identical in all physical properties (R_f, $[\alpha]_D$, IR and ^{1}H NMR) with an authentic sample of tri-*O*-acetyl-β-aucubigenin [9].

The presence in **2**, as in aucubin (**1**), of a β-D-glucose unit at C-1 was proved with the formation, by Li/NH_3 reduction, of compound **8** whose identity with 6,10-bisdeoxyaucubin [10] was demonstrated by comparison (^{1}H NMR) with an authentic sample as well as by further comparison (mmp) of their respective acetyl derivatives.

These data allow the deduction of structure **2** for the β-D-xylosyl derivative of **1** in which the *O*-β-D-xylosyl unit could be linked to C-6 or C-10. Noteworthy is the fact that neither the H-6 nor the 2H-10 ^{1}H NMR resonances of **2** showed glycosidation shifts while in **3** the 2H-10 signal was significantly deshielded (0.24 ppm).

By acetylation in mild conditions **2** was transformed

*Part 28 in the series "Iridoids". For Part 27 see ref. [1].

†The two reports known so far are relative to montinioside [4], containing a xylose and a glucose unit, and opulosides III and IV [5], which are both xylo-allosides.

‡To display this signal the HDO peak was shifted upfield by recording the spectrum at 80°.

into the crystalline octaacetate (peracetate) **9** showing a characteristic paramagnetic shift (0.36 ppm) for the allylic hydroxymethyl signal in the ^{1}H NMR spectrum while the chemical shift of the H-6 resonance appeared practically unaffected. This signal, partially covered by the upfield line of the doublet from the β-D-xylose anomeric proton, was identified by a double resonance experiment. This allowed the location at C-6 of the *O*-β-D-xylosyl unit. A different acetylation effect on these protons was obviously observed in **3** [6].

Full spectroscopic evidence for structure **2** was achieved by comparison of PND and SFORD ^{13}C NMR spectra of **2** and **1** (Table 1). The PND spectrum of **2** showed, in agreement with the molecular formula, twenty carbon lines. The aglycone resonances were easily distinguishable from the glycosylic ones exhibiting shift values corresponding, although slightly shielded, to those of **1**. The only exception was the C-6 resonance (88.65 ppm) whose strong downfield shift reflects the effect of the *O*-xylosidation. This deshielding value (7.29 ppm), analogous to that (7.82 ppm) found in **3** for the C-10, is in agreement with the general rule by which the glycosylation of a hydroxyl group of carbohydrates causes a downfield shift (~8–10 ppm) [11] on the α-carbon and an upfield shift (~3–4 ppm) [12, 13] on the β-carbons. According to this rule the significant upfield shifts observed in **2** for the resonances of C-5 (3.26 ppm) and C-7 (4.07 ppm) further support the location at C-6 of the *O*-β-D-xylosyl unit (an analogous β-effect (3.33 ppm) was observed on the C-8 of **3**).

Table 1. ^{13}C NMR chemical shifts of **1-3** and methyl-β-D-xylopyranoside*

Carbon No.	Compound 1	2	3	xylopyranoside†
C-1	96.29 *d*	94.75 *d*	95.98 *d*	
C-3	140.43 *d*	138.99 *d*	140.39 *d*	
C-4	106.06 *d*	103.94 *d*	106.06 *d*	
C-5	43.26 *d*	40.00 *d*	42.91 *d*	
C-6	81.36 *d*	88.65 *d*	81.49 *d*	
C-7	129.41 *d*	125.34 *d*	132.04 *d*	
C-8	147.57 *s*	147.74 *s*	144.24 *s*	
C-9	47.18 *d*	45.66 *d*	47.33 *d*	
C-10	60.28 t	59.80 t	68.10 t	
C-1′	99.23	97.49	99.12	
C-2′	73.64	73.63	73.80	
C-3′	76.97	77.08	76.98	
C-4′	70.42	70.49	70.70	
C-5′	76.49	76.55	76.71	
C-6′	61.53	61.66	61.73	
C-1″		101.60	102.92	105
C-2″		73.99	74.19	73.9
C-3″		76.68	77.24	76.9
C-4″		70.09	70.70	70.5
C-5″		64.00	76.71	65.9
C-6″			61.73	

*The spectra were recorded in D_2O at 20 MHz. Chemical shifts in ppm from TMS (dioxane (67.4 ppm) was used as internal standard).

†In H_2O [14].

The assignment of the three hemiacetal carbon lines of **2** to the β-D-xylose C-1″ (101.60 ppm), the β-D-glucose C-1′ (97.49) and the aglycone C-1 (94.75) respectively, has been made by comparison with the resonances of the corresponding carbons of aucubin (C-1′, 99.23; C-1, 96.29) (unpublished data) and of methyl-β-D-xylopyranoside (C-1″, 105) [14] (Table 1). The discrepancy between the δ values of C-1″ in **2** and C-1 in methyl-β-D-xylopyranoside could be explained by steric compression for C-1″ at the crowded C-6 position in aucubin. The same effect is seen in 6-*O*-α-rhamnosylcatalpol [15].

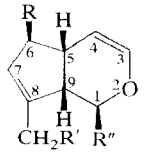
The lines (triplets in SFORD) of the three hydroxymethyl carbons are easily assignable to the β-D-xylopyranose C-5″ (64.00), to the β-D-glucose C-6′ (61.66) and to the aglycone C-10 (59.80) by comparison with the ^{13}C NMR data of **1** and methyl-β-D-xylopyranoside.

The seven remaining glycosylic carbons in the region 78–70 ppm of **2** may be tentatively differentiated by comparison with the ^{13}C NMR data relative to methyl-β-D-xylopyranoside and a large series of iridoid β-D-glucosides (unpublished data). The good agreement between the chemical shifts of methyl-β-D-xylopyranoside and β-D-xylose carbons confirms for the latter the usual [8] pyranose form.

EXPERIMENTAL

Column chromatography was on Si gel 70-230 mesh (Merck) and cellulose CF 11 (Whatman). TLC used Si gel SIF_{254} (Erba) and cellulose (Merck) plates. Paper chromatograms were on Schleicher and Schüll No. 2043 b Mgl paper. Spray reagents: 2 N H_2SO_4, heating at 120° (Si gel plates); vanillin (vanillin 1 g, conc HCl 2 ml, MeOH 100 ml) and benzidine (benzidine 0.5 g, HOAc 20 ml, EtOH 80 ml), heating at 100° (cellulose plates and paper chromatograms). Mps are uncorr. All evapns of volatile material were performed under red. pres.

Isolation of iridoid fraction. Verbascum sinuatum was collected in the neighbourhood of Rome and identified by Dr. Anna L. Francesconi (Botanical Institute, University of Rome). A reference specimen has been deposited at the Botanical Institute Herbarium (University of Rome). Fresh aerial part of the plant (4.0 kg) was extracted 2× with 90% EtOH (7 l. each) at room temp. for 2 days. A paper chromatogram eluted with BuOH–HOAc–H_2O (63:10:27) and visualized with vanillin showed 6 iridoid spots with R_f values: 0.29 (**1**), 0.26 (harpagide), 0.19 (**A**), 0.13 (**2**), 0.05 (**B**), 0.04 (**C**). The ethanolic extract was concd to an aqueous suspension which was washed with C_6H_6 (600 ml) and then



1 R = R′ = OH, R″ = *O*-β-glc
2 R′ = OH, R = *O*-β-xyl, R″ = *O*-β-glc
3 R = OH, R′ = R″ = *O*-β-glc
4 R = R′ = OH, R″ = *O*-β-glc(α1→6)glc
7 R = R′ = R″ = OAc
8 R = R′ = H, R″ = *O*-β-glc
9 R′ = OAc, R = *O*-β-xyl(OAc)$_3$, R″ = *O*-β-glc(OAc)$_4$

treated with charcoal–celite, 5:2 (700 g). The resulting suspension was stratified on a Gooch funnel (20 cm dia) containing a layer of charcoal–celite, 1:1 (100 g). Monosaccharides were eluted with H_2O (40 l.), disaccharides with 5% EtOH (15 l.), **1** and harpagide (fraction I, 4 g) with 10% EtOH (20 l.) and 15% EtOH (10 l.), and finally a mixture of **2**, **A**, **B**, and **C** (fraction II, 8.5 g) with 20% EtOH (10 l.), 25% EtOH (10 l.) and 30% EtOH (10 l.). Fraction II (8.5 g) chromatographed on cellulose (400 g) in BuOH–MeOH–H_2O (70:10:30) (BMW) afforded the following fractions: (a) **1** (200 mg), (b) **A** and **2** (480 mg), (c) **2** (140 mg), (d) **2** and **B** (350 mg), (e) **B** (485 mg), (f) **B** and **C** (500 mg), (g) **C** (150 mg). Fraction (b), rechromatographed on cellulose in BuOH satd with H_2O (BW), gave **A** (250 mg) and **2** (180 mg). Fractions d, e, f, and g, separately rechromatographed on cellulose in BMW, gave **B** (1.0 g), **C** (260 mg) and **2** (50 mg). Fraction I (4 g) chromatographed on cellulose in BMW gave **1** (1.3 g) and harpagide (70 mg).

6-O-β-Xylosylaucubin (**2**). Crystallized from EtOH as needles, mp 192–193°; $[\alpha]_D^{25}$ −85° (H_2O, *c*, 1.0); UV_{max}^{MeOH} nm (log ε): 204 (3.6); IR ν_{max}^{KBr} cm^{-1}: 3400, 2900, 2860, 1655, 1385, 1245, 1165, 1050, 970. (Found:C, 49.95; H, 6.29. $C_{20}H_{30}O_{13}$ requires: C, 50.21; H, 6.23%). ^1H NMR (D_2O): δ 6.38 (*dd*, $J_{3,4}=6.0$, $J_{3,5}=1.5$ Hz, H-3), 5.98 (*bs*, H-7), 5.22 (*d*, $J_{1,9}=5.0$, H-1), 5.17 (*dd*, $J_{3,4}=6.0$, $J_{4,5}=3.7$ Hz, H-4), 4.60 (*bs*, H-6), 4.38 (*bs*, 2H-10), 3.2–2.9 (H-5, H-9).

Acid hydrolysis of **2**. Compound **2** (150 mg), dissolved in N H_2SO_4 (4 ml), was refluxed for 2 hr. Black degradation products were removed by filtration and the acidic soln neutralized with strong anion exchange resin (Ionenaustauscher III Merck), was evapd and the residue chromatographed on Si gel in $CHCl_3$–MeOH (7:3), gave **5** (20 mg) and **6** (15 mg). Direct comparison with authentic samples established the identity of **5** with D-glucose, (^1H NMR superimposable, $[\alpha]_D^{25}$ +54° (H_2O, *c*, 0.3), R_f 0.16 on paper in *t*-amyl alcohol–*n*-PrOH–H_2O (4:1:1:1.5) (APW)) and of **6** with D-xylose (^1H NMR superimposable, $[\alpha]_D^{25}$ +20° (H_2O, *c*, 0.3), R_f 0.28 on paper in APW).

Tri-O-acetyl-β-aucubigenin (**7**). Compound **2** (100 mg), dissolved in H_2O (0.3 ml), was incubated with hepatopancreatic juice of snail (*Helix pomatia*) (0.7 ml) for 8 hr at 33°. The mixture was extracted with EtOAc, the organic soln carefully evapd and the residue immediately acetylated (Ac_2O, Py) for 2 hr at room temp. MeOH was added, after 20 min the soln was evapd and the residue dissolved in EtOAc. The organic soln, washed with H_2O afforded a residue which, chromatographed on Si gel in C_6H_6–Et_2O (8:2), gave **7** (10 mg). Direct comparison with authentic tri-*O*-acetyl-β-aucubigenin [9] [IR and ^1H NMR superimposable, $[\alpha]_D^{25}$ −190° ($CHCl_3$, *c*, 0.1)] established its identity.

6,10-Bisdeoxyaucubin (**8**). Compound **2** (50 mg) was dissolved in absolute EtOH (1 ml) and, maintaining the temp. at −38°, liquid NH_3 (50 ml) was added. Within 3 hr Li (400 mg) was added in small portions until the persistence of a blue colour, then excess Li was decomposed with absolute EtOH and the NH_3 left to evaporate overnight. The residue was dissolved in H_2O (50 ml) and extracted with EtOAc (5× 50 ml); the organic soln was evapd and the residue chromatographed on Si gel in $CHCl_3$–MeOH (8:2) gave **8** (38 mg) identical with 6,10-bisdeoxyaucubin (^1H NMR superimposable). **8** was acetylated (Ac_2O, Py) in the above conditions and the residue chromatographed on Si gel in Et_2O–hexane (1:1) afforded a tetraacetate (20 mg). Direct comparison with authentic 6,10-bisdeoxyaucubin tetraacetate established identity (mp and mmp 134–135°).

Octaacetate (**9**). Acetylation (Ac_2O, Py) of **2** (50 mg) in the above conditions gave a residue which chromatographed on Si gel in Et_2O gave **9** (55 mg); needles from EtOH, mp 145–146°. (Found: C, 52.84; H, 5.79. $C_{36}H_{46}O_{21}$ requires: C, 53.06; H, 5.69%). ^1H NMR ($CDCl_3$): δ 6.21 (*dd*, $J_{3,4}=6.0$, $J_{3,5}=1.6$ Hz, H-3), 5.84 (*bs*, H-7), 4.74 (*bs*, 2H-10), 4.46 (*bs*, H-6), 5.2–5.0 (H-1), 5.1–4.9 (H-4), 3.2–2.7 (H-5, H-9).

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