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6-*O*-α-SINUATOSYLAUCUBIN FROM *VERBASCUM SINUATUM**

ARMANDODORIANO BIANCO, MARCELLA GUISO, CARLO IAVARONE, PIETRO PASSACANTILLI and CORRADO TROGOLO

Centro di Studio per la Chimica delle Sostanze Organiche Naturali del C.N.R., Roma; Istituto di Chimica Organica dell'Università di Roma, Piazzale Aldo Moro, 5, 00185 Roma, Italy

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Key Word Index—*Verbascum sinuatum*; Scrophulariaceae; iridoid glycosides; 3-*O*-β-D-xylopyranosyl-D-galactopyranose (sinuatose); 6-*O*-α-sinuatosylaucubin.

Abstract—From *Verbascum sinuatum*, besides aucubin, harpagide, 6-*O*-β-D-xylopyranosylaucubin and sinuatol (6-*O*-α-L-rhamnopyranosylaucubin), a new iridoid glycoside, sinuatoside, has been isolated and its structure elucidated as 6-*O*-(3-*O*-β-D-xylopyranosyl)α-D-galactopyranosyl aucubin on the basis of spectral data and chemical modifications. For the new disaccharide unit of the latter compound the name sinuatose is proposed.

INTRODUCTION

Recently we reported the structure and configuration of 6-*O*-β-D-xylopyranosylaucubin (**1**, R_f 0.13) [1] and 6-*O*-α-L-rhamnopyranosylaucubin (sinuatol, **2**, R_f 0.19) [2], two iridoid diglycosides isolated from *Verbascum sinuatum* L. (Scrophulariaceae). In addition, aucubin (**3**, R_f 0.29), the major component, harpagide (R_f 0.26) [3] and two polar compounds (R_f 0.05 and 0.04), probably with iridoid skeletons, were isolated. Here we report the structure of the compound with R_f 0.05, which we name sinuatoside (**4**).

Sinuatoside is the first iridoid glycoside containing three different monose units. One of them, D-galactose, has not been found previously in these compounds and it is linked to D-xylose in a new disaccharide.

RESULTS AND DISCUSSION

Sinuatoside, (**4**) was an amorphous compound with molecular formula $C_{26}H_{40}O_{18}$ and $[\alpha]_D - 55.7°$. Its UV (204 nm, $\log\varepsilon = 3.7$) and IR (1645 cm^{-1}) absorptions were typical of an iridoid containing a non-conjugated enol–ether group. The ^1H NMR spectrum of **4** (see Experimental) supported an iridoid structure showing a close relationship with that of aucubin (**3**). The only differences between the spectra of **4** and **3** were: (1) the presence in **4** of three hemiacetal proton signals (δ 5.21, *bs*; 4.76, *d*, $J_{1,2} = 7.0$ Hz; 4.58, *d*, $J_{1,2} = 7.5$ Hz) attributable to the monose units (only one in **3**, δ 4.78, *d*, $J = 7.0$ Hz). The broad singlet at 5.21 was superimposed on the downfield part of the double doublet of H-4 and showed in the expanded spectrum at 300 MHz an ill-defined doublet structure ($J = 3.0$ Hz); the doublets at 4.76 and 4.58 were revealed by shifting the HDO signal to higher field (heating at 85°); (2) the integral value of the glycosidic region which in **4** corresponded to three monosaccharide units (one in **3**); (3) the resonances of the H-5 and H-9 protons which in **4** were partially covered by glycosidic signals.

The acid hydrolysis of **4** carried out in refluxing 1 N H_2SO_4 for ~10 min afforded two different sugars: the first one identical (R_f, α_D, ^1H NMR spectrum) with D-glucose, and the second one (**5**) with an R_f value (0.08) typical of a disaccharide. Further acid hydrolysis of **5** in refluxing 1 N H_2SO_4 for 1 hr afforded two monoses (1:1 ratio) which were identical (R_f, α_D, ^1H NMR spectra) with D-xylose and D-galactose, respectively.

The ^1H NMR spectrum of **5** (90 MHz, D_2O) at the mutarotational equilibrium showed in the hemiacetal region a broad singlet at δ 5.30 (0.4 H) and a broad doublet at 4.63 (1.6 H) with an apparent $J = 7.0$ Hz, attributable to the α and β anomeric protons respectively. This approximate integral ratio, measured by shifting the HDO signal at 85°, indicated that in **5** the anomeric proton involved in the interglycosidic linkage was in the β configuration. The comparison of the spectral data relative to the anomeric protons of sinuatoside (**4**) with those of the corresponding protons of **5** and of its monose units (D-xylose and D-galactose) established an α configuration for the glycosidic linkage of **5** with the aglycone. The sequence of the monose units in the disaccharide could not be established by the ^1H NMR spectrum owing to the close similarity of the spectral parameters known for the anomeric protons of D-xylose (α: 5.26, *d*, $J = 3.1$ Hz; β: 4.65, *d*, $J = 7.4$ Hz) and D-galactose (α: 5.34, *d*, $J = 2.8$ Hz; β: 4.68, *d*, $J = 7.1$ Hz) [4]. However, it was easily proved by the ^{13}C NMR spectrum (Table 1), which at the anomeric equilibrium consisted of 17 lines; the five of highest intensity had chemical shifts corresponding to those of methyl-β-D-xylopyranoside indicating that in **5** the D-xylose unit was linked in the β configuration to the D-galactose.

The twelve lines remaining were assigned by detailed comparison of the ^{13}C NMR spectral data of **5** with those of α- and β-methyl-D-galactopyranosides and the α- and β-forms of D-galactose (Table 1) to the carbons of the α- and β-forms of the reducing D-galactose unit (the β-form was predominant after the mutarotational equilibrium). Further comparison of the chemical shift values of **5** and **4**

* Part 30 in the series "Iridoids". For Part 29 see ref. [2].

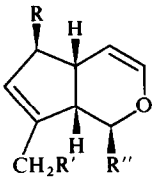
Table 1. ^{13}C NMR chemical shift assignments

Carbon No.	3 [1]	4	5		Methyl-β-D-xylopyranoside*	α-D-Galactopyranose*	Methyl-α-D-galactopyranoside*	β-D-Galactopyranose*	Methyl-β-D-galactopyranoside*
C-1	96.29 *d*	96.00 *d*							
C-3	140.43 *d*	140.53 *d*							
C-4	106.06 *d*	106.16 *d*							
C-5	43.26 *d*	40.63 *d*							
C-6	81.36 *d*	88.00 *d*							
C-7	129.41 *d*	128.13 *d*							
C-8	147.67 *s*	148.84 *s*							
C-9	47.18 *d*	47.08 *d*							
C-10	60.28 *t*	60.26 *t*							
C-1′	99.23	99.23							
C-2′	73.64	73.64	α-D-galacto-	β-D-galacto-					
C-3′	76.97†	77.05†	pyranose	pyranose					
C-4′	70.42	70.44							
C-5′	76.49†	76.56†							
C-6′	61.53	61.62							
C-1″		98.75	92.96	96.97		93.2	100.7	97.5	105.0
C-2″		68.17	68.18	71.77		70.2	70.8	73.0	72.1
C-3″		79.89	79.96	83.07		69.4	71.1	73.8	74.2
C-4″		69.95	69.88	69.24		70.2	69.8	69.7	70.0
C-5″		71.59	71.03	75.63		71.2	71.2	75.9	76.2
C-6″		61.92	61.95	61.76		62.1	62.7	61.9	62.3
C-1‴		105.18	105.20		105.0				
C-2‴		74.04	74.01		73.9				
C-3‴		76.39	76.33		76.9				
C-4‴		69.95	70.03		70.5				
C-5‴		65.92	65.86		65.9				

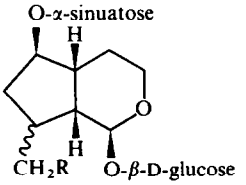
The spectra were recorded in D_2O at 20 MHz. Chemical shifts in ppm from TMS (dioxane 67.4 ppm).

* See ref. [8].

† These signal assignments may be reversed.



1 R = O-β-D-xylose, R′ = OH, R″ = O-β-D-glucose
2 R = O-α-L-rhamnose, R′ = OH R″ = O-β-D-glucose
3 R = R′ = OH, R″ = O-β-D-glucose
4 R = O-α-sinuatose, R′ = OH, R″ = O-β-D-glucose
7 R = R′ = H, R″ = O-β-D-glucose
8 R = O-α-sinuatose $(Ac)_6$, R′ = OAc, R″ = O-β-D-glucose $(Ac)_4$



9 R = OH
10 R = H

allowed the assignment of the α-configuration to the glycosidic linkage in **4** involving the D-galactose unit.

The mean values (~7–10 ppm) usually reported [5] for the downfield shift of the α-carbon due to *O*-glycosidation allowed the elimination of glycosidic linkages at positions C-4 or C-6 of D-galactose but indicated that either C-2 or C-3 may be involved. A conclusive discrimination between these latter positions on the basis of the upfield shift values (~0–4 ppm) [6, 7] of the β-carbons C-1 or C-4 induced by *O*-glycosidation was not possible because in **5** both these carbons show similar small shielding values (from 0.23 to 0.53 ppm) with respect to the corresponding ones of α- and β-D-galactose (Table 1).

In order to establish the linkage point of D-xylose to the D-galactose unit, we treated **5** with acetone dimethylketal–$SnCl_2$. The production of the 1,2-*O*-isopropylidene derivative **6** showed that the xylosyl unit was not linked at C-2 of the D-galactose. The structure of **6** was proved by comparison of its ^{1}H NMR spectrum with that of **5**. The methyl singlets at δ 1.50 and 1.34 confirmed the presence of one isopropylidene unit which was responsible for the downfield shift of 0.61 ppm observed for the signal of the anomeric H-1 of D-galactose. The signal appeared as a well defined doublet (J = 4.3 Hz) owing to the formation of the isopropylidene function between the axial OH groups at C-1 and C-2.

Conclusive chemical evidence for the structure of 3-*O*-β-D-xylopyranosyl-D-galactopyranose was unequivocally obtained by the periodate oxidation of **5** which consumed only 3 mol of $NaIO_4$ for each mole of disaccharide. We propose for this new disaccharide the name sinuatose.

The chemical proof of structure **5** allowed reconsideration of the ^{13}C NMR spectrum and the assignment of the C-2 and C-3 resonances of the α- and β-forms of the D-galactose moiety. The *O*-xylosylation of D-galactose resulted in the expected downfield shift of the C-3 signal (α effect: 10.56 ppm and 9.27 ppm for the α and β forms respectively) in comparison with α- and β-D-galactopyranose.

The production of 6,10-bisdeoxyaucubin (**7**) by Li–NH_3 reduction of **4** demonstrated that the glucose residue was linked in the β-configuration at C-1 of an aucubigenin-type aglycone and that the stereochemistry of the C-1, C-5 and C-9 centres of **4** was identical to that of aucubin. The preparation of **7** proved also that the sinuatose unit must be linked at one of the allylic positions C-6 or C-10.

The acetylation of **4** afforded the hendecaacetyl derivative (peracetate) **8** whose ^{1}H NMR spectrum showed the expected downfield shift for the 10-CH_2 group (0.38 ppm, when compared with **4**).

As no information could be inferred for the H-6 resonance, which was completely submerged in the glycosidic proton region, the linkage of sinuatose to the aucubin C-6 position was definitely proved by hydrogenation of **4** with H_2/Pd–C. Two derivatives **9** and **10** were obtained both containing the sinuatose unit. Their ^{1}H NMR spectra (see Experimental) demonstrated that **9** was the 3,4,7,8-tetrahydro derivative of **4** while **10** showed also the doublet (δ 1.15, J = 7.5 Hz) of the methyl group arising from the allylic hydrogenolysis of the free 10-CH_2OH.

The ^{13}C NMR spectrum of **4** (Table 1) was in complete agreement with the proposed structure. In particular, the C-6 resonance showed, unlike that of H-6, a downfield glycosidation shift with respect to the C-6 of aucubin. The magnitude of this α-effect (+6.64 ppm) was similar to that found for 6-*O*-β-D-xylopyranosylaucubin (**1**) (7.29 ppm) [1] and sinuatol (**2**) (6.91 ppm) [2]. Rather similar values were also observed in the same compounds for the upfield glycosidation shift (β effect) on C-5 (2.63 ppm for **4**, 3.26 ppm for **1** [1] and 1.65 ppm for **2** [2]). As regards the configuration of the OH-6 of **4**, its β-orientation was well supported by the invariance of the C-6 resonance in **4** (88.00 ppm) and in 6-*O*-glycosylaucubins **1** (88.65, [1]) and **2** (88.27, [2]) and also by the identity of the H-6 chemical shift of **4** (δ 4.58) with that (4.60) observed in **1**, **2** and **3**. In fact in 6-epiaucubin [2] the same proton resonates at lower field (Δδ = 0.26 ppm) with respect to aucubin (**3**).

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, eluted with BuOH–HOAc–H_2O (BAW) 63:10:27 (iridoid glycosides) or *t*-amyl alcohol–*n*-PrOH–H_2O (APW) 4:1:1.5 (sugars). Spray reagents: NH 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. Compounds **4** and **8** gave satisfactory elemental analysis.

Isolation of iridoid fraction. Fresh aerial parts of *Verbascum sinuatum* (4.0 kg), collected in the neighbourhood of Rome at summer time and identified by Dr. Anna L. Francesconi (Botanical Institute, University of Rome), was worked up as previously described [1] giving **3** (R_f 0.29, 1.5 g), harpagide (R_f 0.26, 70 mg), **2** (R_f 0.19, 250 mg) [2], **1** (R_f 0.13, 230 mg) [1], sinuatoside (**4**) (R_f 0.05, 1.0 g) and one other unknown iridoid (R_f 0.04, 260 mg).

Sinuatoside (**4**). Amorphous compound; $[\alpha]_D^{25} = -55.7°$ (H_2O, *c*, 1.6); UV λ_{max}^{MeOH} nm (log ε): 204 (3.7); IR ν_{max}^{KBr} cm^{-1}: 3400, 2900, 1645, 1225, 1050; ^1H NMR (90 MHz, D_2O): δ 634 (*dd*, $J_{3,4} = 6.3$, $J_{3,5} = 1.7$ Hz, H-3), 5.97 (*bs*, H-7), 5.36 (*d*, $J_{1,9} = 4.5$ Hz, H-1), 5.20 (*bs*, H-1″), 5.17 (*dd*, partly masked by H-1″ signals, $J_{4,5} = 3.0$ Hz, H-4), 4.76 (*d*, partly covered by HDO signal, H-1′), 4.58 (*bs*, H-6), 4.36 (*bs*, 2H-10).

Acid hydrolysis of **4** *to yield sinuatose* (**5**). Compound **4** (1.8 g) dissolved in 1 N H_2SO_4 (8 ml), was refluxed for 10 min (negative vanillin test). Black degradation products were removed by filtration and the acidic soln neutralized with $Ba(OH)_2$. $BaSO_4$ was eliminated by centrifugation, the soln was stirred with charcoal–celite, 1:1 (20 g; absence of **5** on TLC) and the resulting suspension stratified on a Gooch funnel (7 cm dia.) containing a layer of charcoal–celite, 1:1 (10 g). Elution with H_2O (2.5 l.) afforded a monosaccharide (350 mg, R_f 0.16) which was purified on Si gel in $CHCl_3$–MeOH (7:3) to give material identical with D-glucose by direct comparison (α_D, ^1H NMR) with an authentic sample. Elution with 10% EtOH afforded in the first fractions (200 ml) **5** and residual glucose (25 mg); the successive fractions (2 l, superimposed to H-1‴), eluted **5** (210 mg, R_f 008). Sinuatose (**5**) was an amorphous compound, $[\alpha]_D^{25} = +31.7°$ (H_2O, *c*, 1.6).

Acid hydrolysis of **5**. Compound **5** (100 mg), dissolved in 1 N H_2SO_4 (10 ml), was refluxed for 4 hr. The acidic soln, neutralized with strong anion exchange resin (Ionenaustauscher III Merck), was evapd and the residue (80 mg) chromatographed on Si gel in $CHCl_3$–MeOH (7:3) to give D-xylose (30 mg, R_f 0.28) and D-galactose (35 mg, R_f 0.14). Direct comparison (α_D and ^1H-NMR) with authentic samples established their identities.

1,2-O-*isopropylidensinuatose* **6**. Compound **5** (50 mg) was treated with a 15% soln of $SnCl_2$ in Me_2CO (2 ml) adding acetonedimethylketal (1 ml). The suspension was stirred at room temp. for 20 hr, then poured into cold satd $NaHCO_3$ soln. The resulting suspension was centrifuged washing the residue twice with Me_2CO–H_2O (1:1). The Me_2CO was evapd from the final soln which was treated with decolorizing charcoal (500 mg). The suspension was stratified on a Gooch funnel (2 cm dia.) and the charcoal layer washed with H_2O (500 ml). Elution with 50% EtOH afforded **6** (20 mg) which was purified on Si gel in BuOH satd with H_2O to yield pure **6** (10 mg). ^1H NMR (D_2O): δ 5.90 (*d*, $J = 4.3$ Hz, H-1), 4.50 (*d*, $J = 7.5$ Hz, H-1′), 1.50 and 1.34 (*s*, CH_3).

$NaIO_4$ *Oxidation of* **5**. Compound **5** (42.58 mg) dissolved in H_2O (10 ml) was treated at room temp. with 1 M $NaIO_4$ (40 ml). Samples of 5 ml were used for each titration. Times in min (mol of $NaIO_4$/mol of **5**): 10 (2.5), 20 (2.6), 30 (2.9), 45 (3.0), 60 (2.9).

6,10-*Bisdeoxyaucubin* (**7**). Compound **4** (150 mg) was dissolved in abs EtOH (1 ml) and, keeping the apparatus at −40°, liquid NH_3 (100 ml) was added over 4 hr. Li (500 mg) was added in small portions until a blue colour persisted, then excess Li was decomposed with abs 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) to give **7** (50 mg). Direct comparison with authentic 6,10-bisdeoxyaucubin established identity (^1H NMR spectra superimposable).

Hendecaacetate **8**. Compound **4** (350 mg) was acetylated (Ac_2O–pyridine) for 2 hr at room temp. MeOH was added and after 20 min the soln was evapd and the residue dissolved in EtOAc. The organic soln, washed with H_2O, afforded a residue which was chromatographed on Si gel in Et_2O–EtOAc (92:8) to give **8** (230 mg), crystallized from CCl_4–hexane (1:1), mp 92–94°; $[\alpha]_D^{25} = -29.7°$ (Me_2CO, *c*, 1.6). ^1H NMR ($CDCl_3$): δ 6.18 (*dd*, $J_{3,4} = 6.3$, $J_{3,5} = 1.7$ Hz, H-3), 5.84 (*bs*, H-7), 5.42 (*d*, $J = 3.3$ Hz, H-1″), 4.74 (*bs*, 2H-10), 3.10 (*bs*, H-9), 2.76 (*bs*, H-5), 2.3 – 1.9 (acetyls).

Reduction of **4** *to yield derivatives* **9** *and* **10**. Compound **4** (400 mg) dissolved in 70% EtOH (25 ml) was added to 200 mg of 10% Pd–C previously suspended in EtOH (10 ml) and satd with H_2. H_2 absorption was complete in 30 min at 25°. After removal of the catalyst the soln was evapd affording a residue (400 mg) which was chromatographed on Si gel in $CHCl_3$–MeOH (6:4) giving **9** (185 mg) and **10** (160 mg) both as amorphous powder. ^1H NMR of **9** (D_2O): δ 5.05 (*bs*, H-1″), 4.1 – 3.5 (CH_2OH-8, 2H-3, H-6), 2.6 – 1.1 (2H-4, H-5, 2H-7, H-8, H-9). ^1H NMR of **10** (D_2O): δ 5.08 (*bs*, H-1″), 4.78 (*d*, partly masked by HDO signal, H-1′), 4.64 (*d*, partly masked by HDO signal, H-1‴), 4.2 – 3.5 (2H-3, H-6), 2.8 – 1.2 (2H-4, H-5, 2H-7, H-8, H-9), 1.15 (*d*, $J = 7.5$ Hz, Me-8).

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