AUCUBIGENIN-1-*O*-β-SEROTINOSIDE, A NEW IRIDOID GLYCOSIDE FROM *ODONTITES VERNA* SSP. *SEROTINA*[1]

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ABSTRACT.—*Odontites verna* ssp. *serotina* contains, besides odontoside, 8-epiloganin, mussaenoside, shanzhiside methyl ester, aucubin, catalpol and 10-*O*-β-glucosylaucubin, a new highly polar iridoid glycoside: the aucubigenin-1-*O*-β-serotinoside (**1**). The disaccharide moiety of **1** is a rare sugar which we have named serotinose; it is 6-*O*-α-D-xylopyranosyl-D-glucopyranose and is linked in β configuration to aucubigenin.

Odontites verna ssp. *serotina* is a scrophulariaceous plant widely distributed in Italy. Seven iridoids (odontoside, 8-epiloganin, mussaenoside, shanzhiside methyl ester, aucubin, catalpol and 10-0-β-glucosylaucubin) have been isolated from this plant (1, 2). We describe here a new iridoid diglycoside, the aucubigenin-1-*O*-β-serotinoside (**1**). The 6-*O*-α-D-xylopyranosyl-D-glucopyranose, a rare disaccharide that has never before been detected as an isolated unit (3), is present in **1**. We propose for this glycobiose the name of serotinose. It is noteworthy that **1** is the first iridoid containing the α-D-xylopyranose unit. Previously only β-D-xylopyranose moiety had been found in these compounds (4, 5, 6, 7).

RESULTS AND DISCUSSION

Compound **1** is an amorphous product with the molecular formula of $C_{20}H_{30}O_{13}$ and $[\alpha]^{25}D = -44.7°$. Its acid hydrolysis, carried out by refluxing in 1N H_2SO_4, afforded, besides the black products arising from the aglycone decomposition, two different monosaccharides in the molar ratio of 1:1. They have been identified as D-glucose and D-xylose on the basis of their chromatographic and physical properties. To establish whether these sugars were combined in a glycobiose moiety, the acid hydrolysis was interrupted as soon as the decomposition of the aglycone unit was complete (negative vanillin test). In fact, the acetalic function of iridoids is more easily hydrolyzed than the interglucosidic functions (6, 7). By this procedure we isolated from **1** a disaccharide which proved to be identical to 6-*O*-α-xylopyranosyl-D-glucopyranose. We named this glycobiose "serotinose" as it was found for the first time in *O. verna* ssp. *serotina* (3).

The structure of the aglycone of **1** is like that of aucubigenin as demonstrated by comparison of the ^1H- and ^{13}C-nmr spectra of **1** and aucubin **2** (see experimental and table).

In the comparison of the ^1H-nmr spectra of **1** and **2**, the only noteworthy difference is seen for the signals derived from the sugar moieties. In the spectrum of **2**, only one signal from an anomeric proton is seen (4.78 ppm, $J = 7.0$ Hz), while two such signals are present in the spectrum of **1**. The first, $J = 7.0$ Hz, was at 4.73 ppm, and was assigned to the acetalic proton of the β-glucopyranose unit and indicated that the serotinosyl residue was in β configuration. The second, $J = 3.0$ Hz, was at 4.83 ppm, and was attributed to the acetalic proton of the α-xylopyranose moiety.

This last signal undergoes an upfield shift in respect to the absorption position of the hemiacetalic proton of D-xylopyranose in α configuration (5.26 ppm) (8).

[1]Part III in the series 'Iridoids in the flores of Italy'. For part II see ref. 2.

This upfield shift is analogous to that observed for the interglucosidic acetalic proton of isomaltose (4.99 ppm) (9) and of isomaltosides (see e.g. aucubigenin-1-*O*-β-isomaltoside, 4.99 ppm) (10) in comparison with the hemiacetalic proton of D-glucopyranose in α configuration (5.26 ppm) (9). The α 1→6 interglucosidic bond seemed to be, therefore, characterized in the ^{1}H-nmr spectrum by a noteworthy shielding effect because significant shifts are not observed for the other interglucosidic bonds (9).

In the ^{13}C-nmr spectrum of **1**, the near identity of the aglyconic signals with the corresponding ones of the spectrum of **2** (see table) definitively proves that

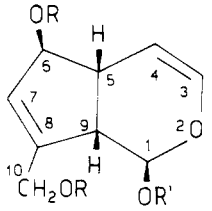
TABLE 1. ^{13}C-nmr chemical shifts of 1, 2 and methyl-α-D-xylose.[1, 2]

Carbon No.	Compounds		methyl-α-D-xylose[3]
	1	**2**	
1	96.4	96.3	
3	140.4	140.4	
4	106.2	106.1	
5	43.5	43.3	
6	81.5	81.4	
7	129.8	129.4	
8	147.4	147.6	
9	47.4	47.2	
10	60.5	60.3	
1'	99.1[a]	99.2	
2'	73.7	73.6	
3'	76.8	77.0[a]	
4'	70.4[b]	70.4	
5'	75.7	76.5[a]	
6'	67.0	61.5	
1''	99.3[a]		99.5
2''	72.4		72.4
3''	74.2		74.4
4''	70.3[b]		70.5
5''	62.3		61.9

[1]Solvent used was D_2O. Standard used was MeOH (49.6 ppm from TMS). Chemical shift in ppm ±0.1.

[2]Values with same superscript in the vertical column are interchangeable.

[3]J. B. Stothers, Carbon-13 NMR Spectroscopy, Academic Press, New York 1972.



1 R=H R'=β-serotinose

2 R=H R'=β-glucose

3 R=Ac R'=β-serotinose $(Ac)_6$

the stereochemistry of the chiral aglyconic centers of **1** and **2** are the same. In regard to the glycosylic signals, they may be easily assigned by combining the ^{13}C-nmr spectrum of **2** with that of methyl-α-D-xylose (see table) and also considering the glycosylation shift (11). The C-6' of glucose, in fact, undergoes a downfield shift of 5.5 ppm, while C-5', which is in β position in respect to the glycosidation side, is slightly shielded of $\sim$1 ppm; these α and β glycosylation shifts are very similar to those reported in other disaccharides (12).

Additional data on the structure of **1** were obtained from the ^{1}H-nmr spectrum of its acetylderivative **3** (see experimental). In fact, in the comparison between the ^{1}H-nmr spectra of **1** and **3**, the H-6 and 2H-10 protons were, as expected, deshielded: The downfield shift of the acetylated glycosylic protons disclosed the 2H-6' signal which absorbed, superimposed with the 2H-5" of xylose, at 3.75 ppm, thus further confirming the (1$\rightarrow$6) interglycosidic bond.

In conclusion, all the reported data allowed the assignment of **1** for the structure and configuration of aucubigenin-1-*O*-β-serotinoside.

EXPERIMENTAL[2]

ISOLATION OF IRIDOIDIC FRACTION.—*Odontites verna* ssp. *serotina* Dumort was collected while it was in flower in October 1979 at the foot of the Monte Mario (Roma, Italy). Voucher specimens of the plant were identified by Dr. Anna Francesconi in the herbarium of Istituto di Botanica dell'Universita di Roma. The fresh aerial part of the plant (4 kg) was extracted twice with 90% ethanol (8 liters each) at room teperature for three days. Paper chromatography in *n*-butanol-acetic acid-water (63:10:27) showed eleven iriodids with Rf: 0.69 (odontoside), 0.58 (mussaenoside), 0.56 (8-epiloganin), 0.40 (shanzhiside methyl ester), 0.30 (I unknown), 0.25 (aucubin **2**), 0.22 (catalpol), 0.11 (aucubigenin-1-*O*-β-serotinoside **1**), 0.09 (II unknown), 0.07 (III unknown), 0.05 (IV unknown). The ethanolic extract was concentrated to an aqueous suspension which was then treated with decolorizing charcoal (1 kg). The resulting suspension was stratified on a Gooch funnel (20 cm dia) containing a layer of charcoal (100 g). Monosaccharides were eluted with H_2O (30 liters), disaccharides with 5% ethanol (5 liters), **2** and the major part of iridoids with lower Rf value with 30% ethanol (25 liters, fraction A), **2** and the major part of iridoids with higher Rf value with 50% ethanol (8 liters, fraction B) and 80% ethanol (8 liters, fraction C). Fraction A (9 g) was chromatographed on cellulose (400 g) in *n*-butanol-methanol-water (70:5:25) and afforded the following fractions: a) **2** (2.5 g); b) catalpol (1.8 g); c) **1** (0.3 g); d) **1** and II (0.1 g); e) II (0.25 g); f) II and III (0.1 g); g) III (0.35 g); h) III and IV (0.12 g); i) IV (0.3 g). Fractions c) and d) were rechromatographed separately on cellulose in *n*-butanol-methanol-water (70:5:25) and gave crude **1** (0.3 g). 10-*O*-β-Glucosylaucubin was eluted together with compound III. These two compounds were separated by acetylation. Chromatographic purification of fractions B and C is described in ref. 2.

OCTAACETYLDERIVATIVE (**3**).—Crude **1** (0.3 g) was treated with dry pyridine (1.0 ml) and acetic anhydride (2.0 ml) for 1.5 hr at rt. After the addition of methanol (6.0 ml), the solution was left for 20 min, then evaporated. The crude **3** (0.4 g) which was thus obtained was then chromatographed on silica gel (30 g) in benzene-t-butyl-methyl ether (1:1). The resulting pure **3** (0.25 g) crystallized from ethanol as needles, mp 228–229°. It gave the following spectral data: ^{1}H-nmr ($CDCl_3$): δ 6.12(1H,dd,$J_{3,4}$=6.0, $J_{3,5}$=2.0 Hz, H-3), 5.85(1H,m,H-7), 5.15(1H,d, $J_{1,9}$=4.5 Hz, H-1), 4.78(2H,bs,2H-10), 4.22(1H,m,H-5'), 3.75(4H,2H-6' and 2H-5"), 3.15(1H, m,H-9), 2.90(1H,m,H-5).

Anal. Calcd for $C_{36}H_{46}O_{21}$: C, 53.06; H, 5.69. Found: C, 52.98; H, 5.73%.

ALKALINE HYDROLYSIS OF **3**, TO GIVE PURE **1**.—Compound **3** (0.25 g) was dissolved in methanol/2N NaOH(1:1) and left at rt overnight. The solution was neutralized by bubbling with CO_2. Methanol was eliminated by evaporation, and then charcoal was added until a negative vanillin test was obtained for the aqueous solution (5.0 g). The suspension was stratified on a

[2]For column chromatography, silica gel, 70–230 mesh, (Merch) and cellulose CF 11 (Whatman) were used. For tlc silica gel SIF_{254} (Carlo Erba) and cellulose plates (Merck) were used. For paper chromatography, Schleicher & Schull, no 2043B Mgl paper was used. The spray reagents were $2NH_2SO_4$, vanillin [vanillin (2 g), hydrochloric acid (4 ml) and methanol (100 ml)]; benzidine (benzidine 0.5 g), acetic acid (20 ml) and ethanol (80 ml)] and resorcin [resorcin (5 g),conc. H_2SO_4 (4 ml), EtOH (296 ml)]. The instruments used were: ^{1}H-nmr, JEOL C-60; ^{13}C-nmr, Varian XL-100; ir, uv, or, Perkin-Elmer 257, 137 and 141, respectively. All evaporation of volatile material was performed under reduced pressure.

Gooch funnel, and the charcoal was washed with H_2O until a negative test for salts was obtained. Then 200 ml of a continuous gradient of ethanol (0→50%) was passed through the filter. Pure **1** was obtained as an amorphous powder (0.15 g). It gave $[\alpha]^{25}D = -44.7°$ (MeOH, c 1.6); λ max (MeOH) = 204 nm (log ε = 3.8); ν max (KBr) = 3350, 2900, 1640, 1420, 1370, 1230, 1050, 1010 cm^{-1}; ^{1}H-nmr (D_2O): δ 6.22(1H,dd,$J_{3,4}$=6.0, $J_{3,5}$=2.0 Hz, H-3), 5.76 (1H,m,H-7), 5.12(1H,d,$J_{1,9}$=4.5 Hz, H-1), 5.02(1H,dd,$J_{4,3}$=6.0, $J_{4,5}$=4.0 Hz, H-4), 4.83(1H,d,$J_{1'',2''}$= 3.0 Hz, H-1''), 4.73(1H,d,$J_{1',2'}$=7.0 Hz, H-1'), 4.45(1H,m,H-6), 4.20(2H,bs,2H-10), 3.02(1H, m,H-9), 2.71(1H,m,H-5).

Anal. Caled for $C_{20}H_{30}O_{13}$: C,50.21; H, 6.23. Found: C, 49.87; H, 6.32%.

TOTAL ACID HYDROLYSIS OF **1**.—Compound **1** (0.1 g) was dissolved in N H_2SO_4 (4 ml) and then refluxed for 6 hr. Black degradation products were removed by filtration, and the acid solution was neutralized with $Ba(OH)_2$ (sat. sol.). After filtration, the solution was evaporated and the residue chromatographed on silica gel in chloroform-methanol (7.3). The 15 mg of D-xylose and 20 mg of D-glucose thus obtained was identified by comparison with authentic samples (Rf, $[\alpha]D$, ir, ^{1}H-nmr).

PARTIAL HYDROLYSIS OF **1**.—Compound **1** (0.1 g) was dissolved in N H_2SO_4 (4 ml) and then refluxed for 10 min until a negative vanillin test was obtained. The solution was rapidly cooled and worked up as above. The neutral solution was treated with charcoal (10 g), and the resulting suspension was stratified on a Gooch funnel and 200 ml of a continuous gradient of ethanol (0→30%) was passed through. Pure 6-*O*-α-D-xylopyranosyl-D-glucopyranose (30 mg) was obtained and identified by comparison of its physical data (mp, $[\alpha]D$) with those reported (3). For an easier comparison with ^{1}H-nmr data of **1** we report also the ^{1}H-nmr (D_2O) of aucubin (**2**): δ 6.20(1H,dd,$J_{3,4}$=6.0, $J_{3,5}$=1.5 Hz, H-3), 5.74(1H,m,H-7), 5.16(1H,d,$J_{1,9}$=5.0 Hz, H-1), 5.01(1H,dd,$J_{4,3}$=6.0,$J_{4,5}$=4.0 Hz, H-4), 4.70(1H,d,$J_{1',2'}$=7.0 Hz, H-1'), 4.46(1H,m, H-6), 4.23(2H,bs,2H-10), 3.03(1H,m,H-9), 2.74(1H,m,H-5).

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