6-O-GLUCOSYL-2'-O-BENZOYLAUCUBIN, A NEW IRIDOID GLUCOSIDE[1]

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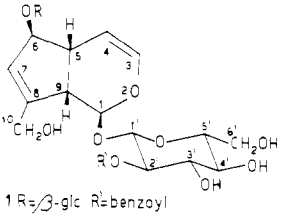
ABSTRACT.—From *Odontites verna* ssp. *serotina*, a new iridoid (**1**) containing two glucose moieties and a residue of benzoic acid has been isolated. The structure **1** and configuration of 6-*O*-β-glucopyranosyl-2'-*O*-benzoylaucubin were based on chemical and spectroscopic data.

Odontites verna ssp. *serotina* contains at least fourteen iridoids (1,2) and it appears as the scrophulariaceous plant which produces the greatest number of these monoterpenes. In this paper we describe the isolation of an iridoid diglucoside esterified by a benzoyl unit, 6-*O*-β-glucopyranosyl-2'-*O*-benzoylaucubin (**1**).



1 R=β-glc R'=benzoyl
2 R=R'=H
3 R=H R'=benzoyl
4 R=β-glc R'=H

RESULTS AND DISCUSSION

Compound (**1**) is a crystalline product with the molecular formula $C_{28}H_{36}O_{15}$. It exhibits a pink-lilac reaction with vanillin reagent identical to that of aucubin (**2**) which is also present in *O. verna*. The ^{1}H-nmr spectrum of **1** (see experimental) confirmed the iridoid structure and indicated that in **1** two units of sugar and one of benzoic acid were present. That was confirmed by the acid hydrolysis of **1**, which afforded two moles of glucose and one of benzoic acid together with the black products arising from the decomposition of the aglycone moiety. In regard to the aglycone part of **1**, the signal pattern of the ^{1}H-nmr spectrum of **1** indicated a close likeness between **1** and 2'-*O*-benzoylaucubin (**3**) (1), an iridoid ester isolated by us from *O. verna*. The presence in **1** of a residue of **3** has been demonstrated by the enzymatic hydrolysis of **1** with β-glucosidase. One mole of **3** together with one mole of glucose was quantitatively obtained by this reaction, demonstrating that **1** was a glucosylderivative of **3**. The glucosylation site has been suggested by the analysis of the ^{13}C-nmr spectrum of **1** in comparison with those of **3** and **2** (see table). It is a well known fact that the carbon bearing the glycosyl residue undergoes a down-field shift (α glycosylation effect, ~ 8 to 10 ppm), while the carbons in β position in respect to the glycosylation site undergo an upfield shift (β glycosylation effect, ~ 2 to 4 ppm) (3). The C-6 carbon appeared in the spectrum of **1** at 89.3 ppm deshielded of 7.9 ppm in respect to the chemical shift value measured in the spectrum of **2** (81.4 ppm) and of 8.8 ppm in respect to that of **3** (80.5 ppm). On the contrary the C-5 and C-7 appeared to be shielded about 3 to 4 ppm. These data suggested that the secondary hydroxyl at C-6 of **1** was the glucosylation site. In this case in **1** a unit of 6-*O*-β-glucopyranosylaucubin (**4**) (4) must be present. Compound (**4**) is an iridoid diglucoside which we isolated, as was **3**, from *O. verna*. The alkaline hydrolysis of **1** afforded, as expected, a

[1]Part VII in the series 'Iridoids in the Flora of Italy'. For part VI see ref. [1].

TABLE 1. ^{13}C-nmr data of **1**–**4** (20 MHz, D_2O).[a]

Carbon No.	Compounds			
	1	**3**	**4**	**2**
1	94.5 (175x)	94.7 (172x)	96.4	96.3 (172x)
3	139.4	139.3	140.5	140.4 (195x)
4	105.8 (165x)	105.9 (164x)	105.8	106.1 (160x)
5	39.6	41.5 (137x)	41.7	43.3 (137x)
6	89.3	80.5 (156x)	90.4	81.4 (156x)
7	126.2	128.7	127.0	129.4 (165)
8	149.9	147.7	149.3	147.6
9	47.1	46.9 (135x)	47.4	47.3 (135x)
10	59.8	59.8 (142)	60.3	60.3 (142)
1'	97.1 (163)	97.3 (162)	99.2	99.2 (162)
2'	74.7	74.7 (152)	73.6[a]	73.6 (145)
3'	74.4	74.4 (144)	76.9[b]	77.0[a] (142)
4'	70.2[a]	70.4 (140)	70.4	70.4 (144)
5'	77.2[b]	77.2 (143)	76.9[b]	76.5[a] (142)
6'	61.4	61.5 (144)	61.5	61.5 (144)
1''	102.4 (162)		102.5	
2''	73.9		74.0[a]	
3''	76.6[b]		76.6[b]	
4''	70.3[a]		70.4	
5''	76.6[b]		76.6[b]	
6''	61.4		61.5	
C=O	168.2	168.0		
1'''	129.9	130.1		
2'''	130.4	130.4		
3'''	129.4	129.3		
4'''	134.7	134.6		
5'''	129.4	129.3		
6'''	130.4	130.4		

[a]The standard used was methanol (49.6 ppm from TMS). Chemical shifts in ppm±0.1. Coupling constants in Hz±2. Mark 'x' indicates that an additional coupling of 3÷5 Hz is present. Values with the same superscript in the vertical column are interchangeable.

very polar iridoid which was identical to **4**. Therefore, the structure and configuration of 6-*O*-β-glucopyranosyl-2'-*O*-benzoylaucubin has been demonstrated to be **1**.

The assignments of all signals of the ^{13}C-nmr spectrum of **1** were made by comparison with the ^{13}C-nmr spectra of **4**, **3** and **2**. It should be noted that in **1**, as in **4** and in all the other 6-*O*-glycosylaucubins (4), there was a significant γ-shielding glucosylation effect on C-8. This carbon belongs to the glucosylated allylic alcohol system (4).

EXPERIMENTAL[2]

ISOLATION OF IRIDOIDIC FRACTION.—*Odontites verna* ssp. *serotina* (Scrophulariaceae) (5) was collected where it was in flower in October 1979 at the foot of Monte Mario (Roma, Italy). Voucher specimens of the plant were identified by Dr. Anna Francesconi in the Herbarium of Instituto di Botanica dell'Università di Roma. The isolation of the iridoidic fraction and the complete separation of each iridoid component are described in reference (4), in which **1** was named "II unknown iridoid". From 4 kg of fresh plant, 0.45 g of pure **1** was obtained: **1** crystallized from ethanol as needles, mp 141–143°; $[\alpha]^{25}D = -165.4°$ (MeOH, c 0.8); ir (KBr): γ max 3340, 2900, 2880, 1720, 1650, 1270, 1120, 1070, 1040, 1030, 900, 710 cm^{-1}; uv (H_2O): λ max 233 nm (log ε=3.9); ^{1}H-nmr (D_2O): δ 5.85 (1H, bs, H-7), 5.65 (1H, dd, $J_{3,4}$=6.0, $J_{3,5}$=1.5 Hz, H-3), 5.30 (1H, d, $J_{1,9}$=3.0 Hz, H-1), 4.8÷5.0 (2H, H-6 and H-1'), 4.0÷4.4 (4H, 2H-10, H-1'', H-2'), 3.00 (1H, m, H-9), 2.60 (1H, m, H-5). Anal.: Calcd. for $C_{25}H_{36}O_{13}$: C, 50.00; H, 5.39. Found: C, 49.91; H, 5.43%.

[2]Column chromatography: Silica gel 70–230 mesh (Merck) and cellulose CF 11 (Whatman). Tlc: Silica gel Kieselgel 60 F_{254} and cellulose (Merck) plates. Pc: Schleicher & Schüll No 2043 b Mgl paper. Spray reagents: 2N H_2SO_4, vanillin (vanillin 2 g, conc HCl 4 ml, MeOH 100 ml); benzidine (benzidine 0.5 g, HOAc 20 ml, EtOH 80 ml); and resorcin (resorcin 5 g, conc H_2SO_4 4 ml, EtOH 300 ml). ^{1}H-nmr Perkin-Elmer R-32. ^{13}C-nmr: Varian CFT-20. Ir, or: Perkin-Elmer 257, 141. Uv: Cary 219.

All evaporations of volatile material were performed under reduced pressure.

ACID HYDROLYSIS OF **1**.—Compound **1** (0.1 g) was dissolved in 1N H_2SO_4 (5 ml) and refluxed for 6 hr. Black degradation products were removed by filtration of the hot suspension, and the solution was extracted with diethyl ether. Evaporation of the organic phase afforded a compound (10 mg) which crystallized from water and was found to be identical to an authentic sample of benzoic acid (ir, ^{1}H-nmr, mp and mixed mp). The aqueous phase was neutralized with $Ba(OH)_2$ (sat. sol.); the suspension was filtered and then evaporated. The residue (50 mg) was chromatographed on silica gel in chloroform-methanol (7:3). This procedure gave 45 mg of D-glucose, which was identified by comparison with an authentic sample (Rf, $[\alpha]_D$, ^{1}H-nmr).

ENZYMATIC HYDROLYSIS OF **1**.—Compound **1** (0.1 g) was dissolved in water (5 ml), and 50 mg of β-glucosidase (EC 3.2.1.21. Fluka) was added. After 16 hr the hydrolysis was complete. The solution was concentrated, and the residue was chromatographed on silica gel in chloroform-methanol (85:15). Sixty mg of **3** was obtained and identified by comparison with an authentic sample $[\alpha]_D$, ^{1}H-nmr).

ALKALINE HYDROLYSIS OF **1**.—Compound **1** (0.1 g) was dissolved in 2N NaOH (5 ml) and left at room temperature overnight. The solution was neutralized by bubbling CO_2 into the solution and charcoal was added (10 g) until the aqueous solution gave a negative vanillin test. The suspension was stratified on a gooch funnel (1 cm ∅), and the charcoal was washed with water until a negative salt test was obtained. Then 150 ml of a continuous gradient of ethanol (0→30%) was passed through the column; the pure **4** (70 mg) which was obtained was identified by comparison with an authentic sample (ir, ^{1}H-nmr).

ACKNOWLEDGMENTS

We are indebted to Dr. Anna Francesconi, Istituto di Botanica dell'Università di Roma, for the identification of the plant material and to Mr. Francesco Piccioni for the accurate measurements of the ^{13}C-nmr spectra.

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