ISOLATION AND CHARACTERIZATION OF PAULOWNIOSIDE, A NEW HIGHLY OXYGENATED IRIDOID GLUCOSIDE FROM *PAULOWNIA TOMENTOSA*

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ABSTRACT.—Investigation of the ethanolic extract of the leaves of *Paulownia tomentosa* (Bignoniaceae) resulted in the isolation of aucubin and of a new iridoid glucoside, paulownioside **1**, whose structure and stereochemistry of 5-deoxy,7-epi-cynanchoside was determined by spectral (^{1}H and ^{13}C-nmr) and chemical procedures.

Paulownia tomentosa is an ornamental plant of the family Bignoniaceae originally from Japan.

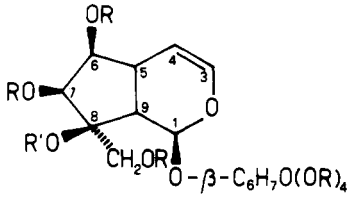
Previous investigation of the species resulted in the isolation of catalpol from the trunk and root bark and, probably, also from the leaves (1).

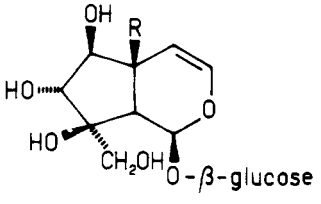
In a recent taxonomic study (2), the isolation from *P. tomentosa* of an "Asarina-glycoside" (identified with macfadyenoside) of another unidentified iridoid and, likely, of aucubin was described.

We undertook consequently the reexamination of the ethanolic extract of leaves of *P. tomentosa* collected in the autumnal season and ascertained the presence of the aucubin (R_f 0.29) in addition to a new highly oxygenated iridoid glucoside (R_f 0.07) which we named paulownioside **1**.

RESULTS AND DISCUSSION

Compound **1** is an amorphous compound with molecular formula of $C_{15}H_{24}O_{11}$, $[\alpha]^{25}D-65°$, and which gave an orange-brown color with iridoid vanillin reagent. It showed significant absorptions at 204 nm (log ϵ 3.2) in the uv spectrum and at 1660 (C=C), 1080 cm^{-1} in the ir (KBr) spectrum in agreement with the presence of a non conjugated enol-ether system.





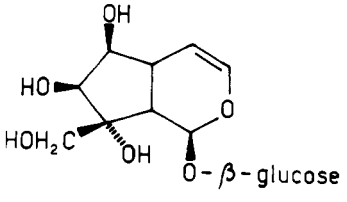
1 R = R' = H

2 R = OH

3 R = Ac, R' = H

5 R = H

4 R = R' = Ac



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Close similarities may be observed (figure 1, table 1) between the ^{1}H-nmr spectra of **1** and cynanchoside **2** (3), except for the presence in **1** of a two-proton broad singlet at δ 2.73 (H-5 and H-9) instead of the one proton singlet at δ 2.45 (H-9) present in **2**. The absence of a hydroxyl function at C-5 of **1** is confirmed by the additional coupling induced on olefinic H-3 and H-4 resonances by H-5 proton.

The enzymatic hydrolysis of **1** with β-glucosidase affording D-glucose (one mole) proved the presence of a β-D-glucopyranosyl moiety linked to the aglycone as supported by the typical doublet (δ 4.75, $J_{1',2'}=7.5$ Hz) of the anomeric H-1'. Assuming the same absolute configuration of C-1, C-5 and C-9 found in all iridoid glucosides (4), the small coupling $J_{1,9}$ (<1 Hz, dihedral angle *ca* 90°) evidences the axial position of the *O*-β-D-glucopyranosyl moiety at C-1.

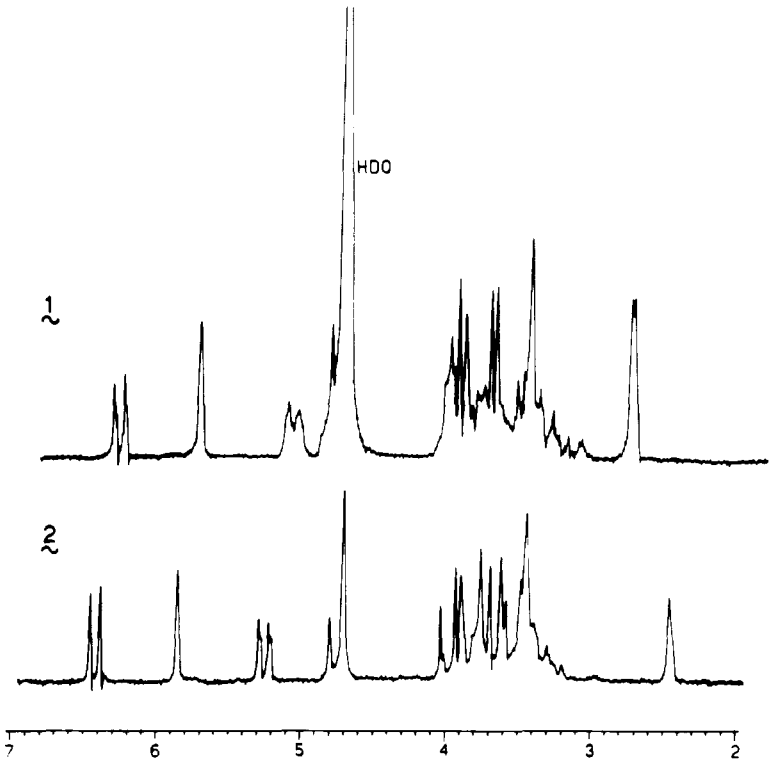


FIG. 1. The ^{1}H-nmr spectra of paulownioside **1** and cynanchoside **2**.

The acetylation of **1** under mild conditions gave the heptaacetate **3**, which still showed OH bands in the ir spectrum (CCl_4). The comparison of the ^{1}H-nmr spectra of **1** and **3** indicated the presence in the aglycone moiety of two secondary and one primary hydroxyl functions. Further acetylation of **3** for 2 days at 40° afforded the octaacetate (peracetate) **4**.

These preliminary data suggested for **1**, apart from the correct configuration of the various chiral centers, a tentative structure of 5-deoxycynanchoside.

The ^{13}C-nmr spectrum (table 2) of **1** (D_2O) confirmed the lack of 5-OH and, although showing different resonance values with respect to **2**, may not be considered in contrast with the above proposed structure.

Comparison of the ^{13}C-nmr spectrum of **1** in CD_3OD with those, in the same solvent, of 10-*des*-cinnamoylglobularimin **5** and 10-*des*-cinnamoylglobularinin **6** (5), indicated that the two latter compounds were not identical with **1**. However a *cis*-relationship was suggested between the 6-OH and 7-OH of **1** from the

Table 1. ^{1}H nmr shift assignments.

Compound	H-1	H-3	H-4	H-5	H-6	H-7	H-9	2H-10	AcO-	Isopr.
1 D_2O	5.72 s	6.28[a] d	5.00[a] d	2.73 bs	3.69 d	3.95 d	2.73 bs	3.93 3.60 AB		
		$J_{3,4}=6.0$ $J_{3,5}=1.0$	$J_{4,5}=1.5$		$J_{6,7}=4.5$			$J_{AB}=13.0$		
2[b] D_2O	5.86 s	6.42 d	5.26 d		3.97 d	3.65 d	2.45 s	3.81 3.57 AB		
		$J_{3,4}=6.0$			$J_{6,7}=9.0$			$J_{AB}=13.0$		
3 $CDCl_3$	5.54 s	6.20 dd	5.1 4.8	2.82 bsg	5.3–4.8	5.3–4.8	2.82 bsg	4.22 bs	2.1–1.9	
		$J_{3,4}=6.0$ $J_{3,5}=2.0$								
4 $CDCl_3$	5.88 s	6.22 dd	4.86[c] bd	2.75[d] bd	5.3–4.9	5.48 d	3.12 bd	4.5–4.0	2.10 1.99 2.04 1.97 2.01	
		$J_{3,4}=6.0$ $J_{3,5}=2.8$		$J_{5,9}=9.0$	$J_{6,7}=4.5$					
7 D_2O	5.53 s	6.21 dd	4.94 bd	2.60 bd	4.2–3.2	4.2 3.2	2.78 bd	4.2–3.2		1.43 1.37
		$J_{3,4}=6.0$ $J_{3,5}=1.0$		$J_{5,9}=9.0$						
9 $CDCl_3$	5.43 s	6.15 dd	4.87 bd	2.67 bd	5.3–4.8	5.3–4.8	2.98 bd	4.22 3.74 AB	2.12 2.01 2.07 1.99 2.05 2.03	1.52 (6H)
				$J_{5,9}=10.0$				$J_{AB}=10.0$		
8 D_2O	5.44 s	6.22 dd	4.81 bd	2.60 bd	4.35 d	4.44 d	2.84 bd	4.09 3.75 AB		1.44 1.41 1.34 1.30
		$J_{3,4}=6.0$ $J_{3,5}=3.0$		$J_{5,9}=9.0$	$J_{6,7}=5.0$			$J_{AB}=10.0$		
10 $CDCl_3$	5.37 s	6.14 dd	4.70 bd	2.55 bd	4.23 s	4.23 s	3.04 bd	4.08 3.58 AB	2.08 2.01 1.99 1.97	1.49 (6H) 1.37 (3H) 1.29 (3H)
		$J_{3,4}=6.0$ $J_{3,5}=3.0$		$J_{5,9}=9.0$				$J_{AB}=10.0$		

[a]In expanded scale (300 Hz) the signal multiplicity of H-3 appears as a doublet of doublets and that of H-4 as a doublet of triplets.
[b]From ref. (3).
[c]Partly overlapped to hydroxymethine protons signals.
[d]With additional fine structure.

TABLE 2. ^{13}C nmr chemical shifts.

Compound	1 D_2O	1 CD_3OD	2[a] D_2O	3[b] $CDCl_3$	5[c] CD_3OD	6[c] CD_3OD	8 D_2O
C-1	93.18 d	93.36 d	91.69 d	91.08 d	93.34	95.16	90.12 d
C-3	140.06 d	141.11 d	140.55 d	140.21 d	140.39	141.61	140.77 d
C-4	105.21 d	105.38 d	109.98 d	103.48 d	106.54	105.27	103.33 d
C-5	35.47 d	37.21 d	64.95 s	33.48 d	37.32	37.16	34.39 d
C-6	74.75 d	75.37 d	82.53 d	74.24 d	83.14	78.34	83.29 d
C-7	76.97 d	78.20 d	78.90 d	75.86 d	86.42	79.34	83.48 d
C-8	79.81 s	80.37 s	76.27 s	77.33 s	80.33	81.03	87.43 s
C-9	48.18 d	49.40 d	56.16 d	48.02 d	48.02	43.70	41.54 d
C-10	65.64 t	66.58 t	62.11 t	67.57 t	64.29	66.37	68.44 t
Me							109.91 s
>*C*							103.40 s
Me							26.98 q
Me							26.13 q
>C							25.94 q
Me							24.26 q
C-1'	98.84	99.51	98.86	95.51			99.05
C-2'	73.48	74.80	73.35	70.78			73.51
C-3'	76.40	78.04	76.27	72.27			76.46
C-4'	70.45	71.68	70.60	68.35			70.47
C-5'	76.97	78.04	77.05	72.64			76.98
C-6'	61.56	62.80	61.63	61.89			61.54

[a]From ref. (3).
[b]Additional signals from acetoxy groups at 170.41, 170.13 ppm (C=O) and 20.76 ppm (CH_3).
[c]From ref. (5).

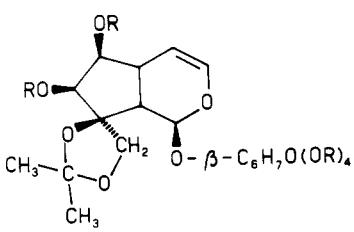
rather shielded values observed for the chemical shifts of carbons -6 and -7 as observed in **6** (6).

The same comparison of spectra gave evidence that the stereochemistry of the C-8 center must be identical in **1** to that (β OH, α CH_2OH) of **5** since there was good agreement between the C-9 resonance values in both compounds (6,3).

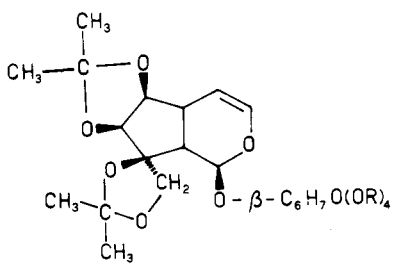
Conclusive chemical evidence for the *cis* arrangement of 6-OH and 7-OH of **1** was achieved by the reaction of **1** with acetone dimethylketal—$SnCl_2$ which afforded the two *O*-isopropylidenderivatives **7** and **8**. The ^1H-nmr spectrum of **7** clearly showed the presence of one isopropylidene group which involved the hydroxyl functions at vicinal C-8 and C-10, as proved by the paramagnetic shifts ($\Delta\delta\simeq1.3$ ppm) observed in its hexaacetate (peracetate) **9** for the resonances of H-6 and H-7.

The easy formation of such a rather unusual 8,10-mono-*O*-isopropylidene derivative was first reported for **2** (3), in that case too as the unique mono-*O*-isopropylidene derivative. The second derivative **8** contained two isopropylidene groups.

The ^{13}C-nmr spectrum of **8** confirmed the location of the isopropylidene units at C-6/C-7 and C-8/C-10 as indicated by the significant deshielding values



7 R = H
9 R = Ac



8 R = H
10 R = Ac

observed for the carbons involved in the ketal functions: C-6 ($\Delta\delta = +8.54$), C-7 ($\Delta\delta = +6.51$), C-8 ($\Delta\delta = +7.62$), C-10 ($\Delta\delta = +2.80$).

In regard to the absolute stereochemistry of the C-6 and C-7 chiral centers, we propose a β configuration for the stated *cis*-diol function on the basis of the following considerations:

1) the non-sharpening of the doublet of H-7 owing to the irradiation of the H-5 signal excludes any long range W coupling (1–1.5 Hz) (5,7) between these protons and, therefore, it excludes a *cis* relationship between βH-5 and H-7. So vicinal 6-OH and 7-OH must both be in β configuration. In fact, in the ^{1}H-nmr spectrum of the octaacetate **4** the H-7 proton resonated as a sharp doublet at δ 5.48. Repeated irradiations of H-5 signal did not produce, even in expanded scale (300 Hz), a significant sharpening of the doublet of H-7, which permits the deduction of α orientation of H-7;

2) the broad doublet of the H-5 resonance of **4**, considering the large direct coupling constant $J_{5,9} = 9.0$ Hz, entailed a predictable small value for the coupling $J_{5,6}$ which demanded a dihedral angle close to 90° between βH-5 and H-6 and, therefore, a *trans* relationship between these protons;

3) the likeness of C-6 and C-7 resonance values in ^{13}C-nmr spectrum of **1** with those of **6** and phlomiol (8) (βOH-6, βOH-7) and the disagreement with those of cynanchoside **2** (3) and 7α-hydroxyharpagide (8) (βOH-6, αOH-7).

Paulownioside **1** must be, therefore, assigned the structure of 5-deoxy-7-epicynanchoside, the C-8 epimer of **6**.

The availability of the ^{13}C data of this new couple of C-8 epimers (**1** & **6**) provided another check of the "C-8 epimer rule" based on the dependence of C-9 resonance from the configuration of the hydroxylated C-8 quaternary carbon (6).

EXPERIMENTAL[1]

PLANT MATERIAL.—Leaves of *Paulownia tomentosa* (Bignoniaceae) were collected in October 1979 from a plant grown in the Botanical Garden of the University of Rome. A reference specimen has been deposited in the herbarium of the same Botanical Institute (Voucher A-33/56).

EXTRACTION AND SEPARATION OF IRIDOID FRACTION.—Fresh leaves of *P. tomentosa* (1.5 kg) were roughly chopped and extracted twice with 90% ethanol (5 liters each) at room temperature for 3 days. The combined ethanolic solutions were concentrated to 0.8 liters and extracted with petroleum ether (bp 40–70°) on a continuous extraction apparatus. A chromatogram on paper, eluted with *n*-butanol-acetic acid-water (63:10:27) and visualized with vanillin, showed 3 spots with R_f 0.29 (aucubin, pink-lilac), 0.18 (sugar compound *A*, pink) and 0.07 (paulownioside, **1**, orange-brown). The aqueous suspension was treated with charcoal (1 kg) and stratified on a layer of silica gel in a gooch funnel (20 cm). Elution with water (12 liters), 5% ethanol (10 liters) and 10% ethanol (10 liters) removed mono- and di-saccharides.

After the beginning of a positive reaction with vanillin reagent, the following fractions were successively eluted: (a) 15% ethanol (5 liters) and 30% ethanol (8 liters): aucubin, compound *A* and paulownioside **1**; (b) 50% ethanol (5 liters) and 80% ethanol (6 liters): aucubin. Fraction (a), evaporated *in vacuo* left an amorphous residue which weighed 6 g; fraction (b), similarly treated, gave 4.5 g of residue.

ISOLATION OF PAULOWNIOSIDE (**1**).—Fraction (a) (6 g) was chromatographed on cellulose (250 g). Elution with *n*-butanol saturated with water (BW) gave, in the first fractions, aucubin (1 g), followed by *A* (150 mg) and finally paulownioside **1** still contaminated by *A* (500 mg). The latter fraction, twice rechromatographed on silica gel in *n*-butanol-methanol-water (7:1:3) and in dichloromethane-ethanol-water (6:4:0.2), respectively, gave pure paulownioside

[1]Column chromatography was performed on silica gel 70–230 mesh (Merck) and cellulose CF 1 (Whatman). Tlc was performed with silica gel SIF_{254} (Erba) and cellulose (Merck) plates. Paper chromatograms were done on Schleicher and Schüll No. 2043 b Mgl paper. Spray reagents: 2N sulfuric acid followed by heating the silica gel plate at 120°; vanillin (vanillin 1 g, 37% hydrochloric acid 2 ml, methanol 100 ml) and 3,5-DNSA (3,5-dinitrosalicylic acid 0.5 g, sodium hydroxide 4 g, water 100 ml), followed by heating the cellulose plates and paper chromatograms at 100°. The ir spectra (KBr disc) were recorded on a Perkin-Elmer 257 and uv spectra on a Perkin-Elmer 137 spectrophotometers. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. ^{1}H-nmr spectra were registered with a Perkin-Elmer R32 (90 MHz) instrument with TMS as the internal reference for the spectra run in $CDCl_3$ and HDO signal (δ 4.70 from TMS) for those in D_2O. Chemical shifts were expressed in δ (ppm downfield from TMS) and J in Hz. ^{13}C-nmr spectra, determined at 20 MHz on a Varian CFT-20 Fourier Transform spectrometer, were referred to the carbon signal of dioxane (67.4 ppm) and computer-converted to δ values from TMS.

1 (370 mg) as a hygroscopic amorphous powder; $[\alpha]^{25}D-65°$ (*c* 2.5, MeOH); uv, λ max (MeOH) 204 nm (log ε 3.2); ir, ν max 3400, 2920, 1660, 1080 and 1020 cm^{-1}. Anal. Calcd. for $C_{15}H_{24}O_{11}$: C, 47.37; H, 6.36. Found: C, 47.20; H, 6.41%.

ACETYLATION OF PAULOWNIOSIDE (**1**).—Paulownioside (**1**, 80 mg) was reacted with pyridine (1 ml) and acetic anhydride (2 ml) at room temperature for 2 hours. After addition of methanol (6 ml), the solution was left for 30 minutes and then evaporated *in vacuo* to dryness. The residue chromatographed on silica gel in ethyl ether gave pure heptaacetate **3** (69 mg); $[\alpha]^{25}D-114°$ (*c* 1.9, MeOH). Anal. Calcd. for $C_{29}H_{38}O_{18}$: C, 51.61; H, 5.68. Found: C, 51.40; H, 5.73%.

ACETYLATION OF **3** TO OCTAACETATE **4**.—Heptaacetate **3** (69 mg) was treated as above at 40° for 2 days. The residue from the usual work-up when chromatographed on silica gel in ethyl ether yielded pure octaacetate **4** (50 mg). Anal. Calcd. for $C_{31}H_{40}O_{19}$: C, 51.95; H, 5.63. Found: C, 51.83; H, 5.70%.

PREPARATION OF 8,10-MONO-*o*-ISOPROPYLIDENPAULOWNIOSIDE (**7**) AND 6,7-8,10-BIS-*o*-ISOPROPYLIDENPAULOWNIOSIDE (**8**).—Paulownioside (**1**, 120 mg), suspended in 0.5 ml of dry acetone, was treated with a solution of anhydrous $SnCl_2$ in dry acetone (250 mg in 2 ml) and with 0.05 ml of acetone-dimethylketal. The suspension was stirred for 2.5 hours at room temperature and then poured into a cold saturated solution of $NaHCO_3$ (100 ml). The obtained suspension was centrifuged, and the residue was washed with acetone-water (1:1). The collected solutions showed, on tlc in BW, the presence of two compounds with higher R_f than **1**. The solvent was evaporated, and the residue, chromatographed on silica gel in BW, afforded **7** (10 mg) and **8** (22 mg) as hygroscopic amorphous powders.

ACETYLATION OF **7**.—8,10-Mono-*O*-isopropylidenpaulownioside (**7**, 10 mg) was treated with pyridine (0.3 ml) and acetic anhydride (0.6 ml) at room temperature for 40 minutes. After the usual work-up, the product was chromatographed on silica gel in benzene-ethyl ether (1:1); pure hexaacetate **9** (12 mg) was obtained.

ACETYLATION OF **8**.—6,7-8,10-Bis-*O*-isopropylidenpaulownioside (**8**, 22 mg) was treated with pyridine (0.4 ml) and acetic anhydride (0.8 ml) at room temperature for 1 hour. The residue from the usual work-up, chromatographed on silica gel in benzene-ethyl ether (7:3) gave pure tetraacetate **10** (23 mg).

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