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CYNANCHOSIDE, A HIGHLY OXYGENATED IRIDOID GLUCOSIDE FROM *MACFADYENA CYNANCHOIDES*

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Key Word Index—*Macfadyena cynanchoides*; Bignoniaceae; iridoid glucoside; cynanchoside.

Abstract—The elucidation of the structure and stereochemistry of cynanchoside, a new highly oxygenated iridoid glucoside isolated from *Macfadyena cynanchoides* (Bignoniaceae), has been accomplished using mainly ^1H and ^{13}C NMR spectral data and further confirmed by simple chemical transformations.

INTRODUCTION

Re-investigation of the iridoid fraction present in the ethanolic extract of leaves of *Macfadyena cynanchoides* (Bignoniaceae), collected in the autumn, revealed the presence, in addition to macfadyenoside (**1**) [1] of at least five other compounds with a possible iridoid structure. In this paper we report the structure of the most polar compound, which was present in small amounts and which we have named cynanchoside (**2**).

RESULTS AND DISCUSSION

Compound **2** was an amorphous compound with molecular formula $C_{15}H_{24}O_{12}$ and $[\alpha]_D - 126°$, which gave a brown colour with the vanillin reagent. Its UV (204 nm, log $\varepsilon = 3.6$) and IR (1670 cm^{-1}) absorptions indicated the presence of a non-conjugated iridoid enol–ether system. By enzymatic hydrolysis with β-glucosidase, **2** gave D-glucose (1 mol) thus permitting the identification of the compound as a β-D-glucopyranoside.

The low R_f value of **2** was typical of highly polar iridoids. Its colour reaction and the co-occurrence of **1** in the plant as the major iridoid component suggested at first the possible identity of **2** with calycinoside (**3**) [2] (5-*O*-β-D-glucosylmacfadyenoside, R_f 0.04, brown reaction with vanillin). However, unlike **1** and **3**, compound **2** did not give a positive Ross test [3] for the oxirane function.

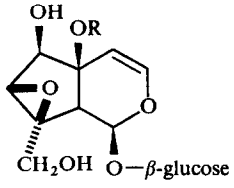
The ^1H NMR spectrum of **2** (Table 1), which showed the typical doublet for the anomeric proton of a β-glucopyranosyl moiety (δ 4.76, $J = 7.5$ Hz), closely resembled that of 7α-hydroxyharpagide (**4**) [4] except for the presence of an AB system for 10-CH_2OH ($\delta_A = 3.81$, $\delta_B = 3.57$, $J_{AB} = 13.0$ Hz) instead of the sharp singlet at δ 1.05 of the methyl group (C-10) in **4**.

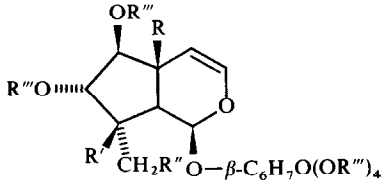
Assuming the same absolute configurations at C-1, C-5 and C-9 as found in other iridoid glucosides [5], the small coupling constant $J_{1,9}$ (*ca* 0 Hz, dihedral angle *ca* 90°) provided evidence for an axial position of the β-D-glucopyranosyl moiety at C-1.

Acetylation of **2** under mild conditions afforded the heptaacetate **5** whose ^1H NMR spectrum exhibited, in comparison with that of **2**, paramagnetic shifts in agreement with the presence in the aglycone moiety of one primary ($\Delta\delta \simeq 0.5$) and two secondary ($\Delta\delta \simeq 1.2$) alcohol functions.

By further acetylation, **5** was completely transformed into the octaacetate **6** owing to the acetylation of one of the two tertiary OH functions (OH-8). In fact **6** showed a residual hydroxyl absorption (OH-5) in the IR spectrum, in agreement with the different reactivity of tertiary OH functions at C-5 and C-8 towards acetylation, which represents a characteristic feature of iridoid glucosides [5].

The structure 7α,10-dihydroxyharpagide suggested for **2** was strengthened by these data and conclusively





1 R = H
3 R = β-glucose

2 R = R′ = R″ = OH, R‴ = H
4 R = R′ = OH, R″ = R‴ = H
5 R = R′ = OH, R″ = OAc, R‴ = Ac
6 R = OH, R′ = R″ = OAc, R‴ = Ac
7 R = R‴ = H, R′ = R″ = OH

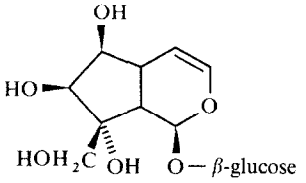
Table 1. ^{1}H NMR shift assignments (90 MHz, δ values)

Compound	H-1	H-3	H-4	H-6	H-7	H-9	2H-10 3H-10	AcO–	Isopr.
2 (D_2O)	5.86 *s*	6.42 *d*	5.26 *d*	3.97 *d*	3.65 *d*	2.45 *s*	3.81 } AB 3.57 }		
		$J_{3,4}$ = 6.0		$J_{6,7}$ = 9.0			J_{AB} = 13.0		
4* (D_2O)	5.61 *s*	6.30 *d*	5.15 *d*	3.81 *d*	3.50 *d*	2.37 *s*	1.05 *s*		
		$J_{3,4}$ = 6.5		$J_{6,7}$ = 9.0					
5 ($CDCl_3$)	5.62 *s*	6.22 *d*	5.44 *d*	4.8–5.3	4.8–5.3	2.88 *s*	4.30 } AB 4.06 }	1.8–2.1	
		$J_{3,4}$ = 6.5					J_{AB} = 12.0		
6 ($CDCl_3$)	5.60 *s*	6.28 *d*	4.8–5.3	5.65† *d*	4.9–5.6	3.54 *s*	4.03 $\frac{1}{2}$AB	1.9–2.2	
		$J_{3,4}$ = 6.3		$J_{6,7}$ = 9.0			J_{AB} = 12.0		
9 (D_2O)	5.71 *s*	6.42 *d*	5.21 *d*	3.97‡ *d*	3.25‡ *d*	2.31 *s*	3.93 *s*		1.33 1.24
		$J_{3,4}$ = 6.5		$J_{6,7}$ = 9.0					
10 (D_2O)	5.66 *s*	6.52 *d*	5.26 *d*	4.05§ *d*	4.23§ *d*	2.66 *s*	4.06 AB		1.50 (3H) 1.39 (9H)
		$J_{3,4}$ = 6.0		$J_{6,7}$ = 6.0			J_{AB} = 12.0		
11 ($CDCl_3$)	5.61 *s*	6.21‡ *d*	5.39‡ *bd*	5.65‡ *d*	4.51‡ *d*	2.76 *s*	3.97 *bs*	2.12,2.08 2.05,2.01	1.38 1.27
		$J_{3,4}$ = 6.3		$J_{6,7}$ = 8.5					
12 ($CDCl_3$)	5.57 *s*	6.34‡ *d*	4.98‡ *d*	3.93‡ *d*	5.44‡ *d*	2.79 *s*	3.98 *bs*	2.14,2.09 2.05,2.00 1.98	1.49,1.36 1.31,1.26
		$J_{3,4}$ = 6.3		$J_{6,7}$ = 6.0					

* These data are relative to a new spectrum we registered to complete those previously reported.
† The high field line of this doublet is overlapped by the H-1 signal.
‡ Assignment checked by spin decoupling experiments.
§ These assignments could be reversed.

confirmed by the ^{13}C NMR spectrum of **2** (Table 2) whose resonances were practically coincident with those of **4** apart from the presence in **2** of a triplet (SFORD) at 62.11 ppm (10-CH_2OH) instead of the corresponding quartet arising from the methyl group in **4**. This coincidence points to the same stereochemistry for all chiral centres in **2** and **4**. In particular, the *trans*-1,2-diol arrangement of the hydroxyl functions at C-6 and C-7 of **2** was well supported by the rather low field value [7] of their resonances, as found also in **4**.

The stereochemistry of the C-8 centre was inferred to be identical in **2** and **4** taking into account the differing effects of an 8α-OH and an 8β-OH substituent upon the resonance of C-9. In fact, it has been observed [7] in the pair of C-8 isomers 10-*des*-cynnamoylglobularimin (**7**) and 10-*des*-cynnamoylglobularinin (**8**) that the β-configuration of OH-8 causes the deshielding of C-9 (δ 48.04) as compared to its α-counterpart (δ 43.70). Analogous effects have been noticed [8] in another pair epimeric at the quaternary C-8, gardenoside (β OH-8, C-9 δ 52.40) and monotropein methyl ester (α OH-8, C-9 δ 45.42).



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Since **7** may be considered a 5-deoxy derivative of **2**, we have calculated from suitable pairs of iridoids, differing only by the presence of the OH-5, that the deshielding effect of the OH-5 on the C-9 resonance is approximately 7 ppm (7.21 ppm in hastatoside [9] and verbenalin [9]; 7.54 ppm in macfadyenoside [9] and catalpol (unpublished results). The subtraction of this shift increment from the resonance of C-9 (δ 56.16) of **2** gives a resonance value in better agreement with that of C-9 of **7** (δ 48.04) than with that of C-9 of **8** (δ 43.70), indicating an identical stereochemistry of the C-8 centre in **2**, **4** and **7**.

Conclusive chemical evidence for the relative orientation of the secondary and tertiary hydroxyl groups in the aglycone moiety of **2** was obtained by reaction of **2** with acetonedimethylketal–$SnCl_2$, which afforded the *O-iso*-propylidene derivatives **9** and **10**.

The ^{1}H NMR spectrum of **9** compared with that of **2** shows the signals of only one *O-iso*-propylidene group (methyl singlets at δ 1.33 and 1.24) and small shift changes for H-6, H-7, H-9 and 10-CH_2 which therefore do not represent useful clues for placing the *iso*-propylidene unit.

A comparison of the ^{13}C NMR spectrum of **9** with that of **2** reveals a large downfield shift for the C-8 of **9** ($\Delta\delta$ = +8.20), analogous to the α-effect caused by the acetylation of a tertiary OH function, and smaller and different effects on C-10 ($\Delta\delta$ = +1.57), C-6 ($\Delta\delta$ = −4.01), C-9 ($\Delta\delta$ = −2.44) and C-7 ($\Delta\delta$ = −1.46).

The acetylation of **9** led to the hexaacetate **11** whose ^{1}H NMR spectrum showed notable downfield shifts for H-6 and H-7 ($\Delta\delta$ 1.68 and 1.26, respectively) showing

Table 2. ^{13}C NMR chemical shifts*

Compound	2 (D_2O)	4† (D_2O)	5‡ ($CDCl_3$)	7§ (CD_3OD)	8§ (CD_3OD/DMSO)	9 (D_2O)	10 (D_2O)
C-1	91.69 *d*	92.01 *d*	91.31 *d*	93.34	95.16	91.32 *d*	91.41 *d*
C-3	140.55 *d*	139.96 *d*	139.74 *d*	140.39	141.61	140.14 *d*	143.54 *d*
C-4	109.28 *d*	109.48 *d*	110.06 *d*	106.54	105.27	109.38 *d*	105.96 *d*
C-5	64.95 *s*	64.17 *s*	64.45 *s*	37.32	37.16	64.15 *s*	75.79 *s*
C-6	82.53 *d*	82.64 *d*	83.98 *d*	83.14	78.34	78.52 *d*	87.71 *d*
C-7	78.90 *d*	78.92 *d*	78.32 *d*	86.42	79.34	77.44 *d*	80.19 *d*
C-8	76.27 *s*	74.91 *s*	75.41 *s*	80.33	81.03	84.47 *s*	88.87 *s*
C-9	56.16 *d*	56.36 *d*	56.83 *d*	48.04	43.70	53.72 *d*	52.26 *d*
C-10	62.11 *t*	16.69 *q*	63.47 *t*	64.29	66.37	63.68 *t*	63.88 *t*
Me>C<							115.33 *s*
Me						110.17 *s*	110.26 *s*
							28.25 *q*
							27.72 *q*
Me–C						26.67 *q*	26.38 *q*
						25.70 *q*	25.88 *q*
C-1′	98.86	98.74	95.89			98.92	99.03
C-2′	73.35	73.36	71.30			73.35	73.25
C-3′	76.27	76.28	72.24			76.28	76.16
C-4′	70.60	70.61	68.37			70.59	70.59
C-5′	77.05	77.06	72.24			76.86	76.85
C-6′	61.63	61.63	61.71			61.43	61.44

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

† From ref. [9].

‡ Additional signals from acetoxy groups at 172.55, 170.68, 170.01, 169.33 ppm (C=O), 21.00 and 20.69 ppm (Me).

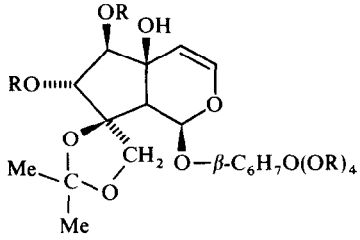
§ From ref. [7].

acetylation at these centres. This clearly confirmed that the ketal ring of **9** must be located at C-8 and C-10 and that this position, new in iridoids, is evidently preferred to that at C-5 and C-6.

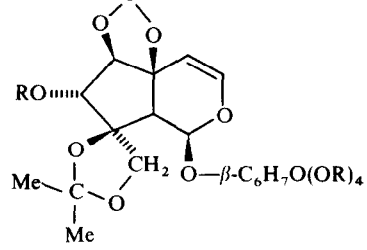
The ^1H NMR spectrum of the second *O-iso*-propylidene derivative **10** showed that it contained two *iso*-propylidene units (methyl singlets at δ 1.50 (3 H) and 1.39 (9 H) respectively). The comparison of the ^{13}C NMR spectrum of **10** with the spectra of **9** and **2** respectively, revealed significant deshielding values for the carbons involved in the ketal functions: C-5 ($\Delta\delta = +11.64$; $+10.84$), C-6 ($\Delta\delta = +9.19$; $+5.18$), C-8 ($\Delta\delta = +4.40$; $+12.60$) and C-10 ($\Delta\delta = +0.20$; $+1.77$). As for the remaining carbons, the upfield shift of C-4 ($\Delta\delta = -3.42$; -3.32) was notable while the C-3 ($\Delta\delta = +3.40$; $+2.99$) and C-7 ($\Delta\delta = +2.75$; $+1.29$) resonances were slightly deshielded. As expected, the acetylation of **10** led to the pentaacetate (peracetate) **12** whose ^1H NMR spectrum showed four distinct methyl singlets and a downfield shift for H-7 ($\Delta\delta$ 1.21). All these data established a 5,6-8,10-*bis*-*O-iso*-propylidene derivative structure for compound **10**.

The ^{13}C NMR spectra of these *O-iso*-propylidene derivatives of iridoid glucosides, which are the first to be reported, allow it to be concluded that carbons bearing primary and tertiary hydroxyl functions are slightly (*ca* 1–2 ppm) and strongly (*ca* 8–12 ppm) deshielded, respectively, when involved in the formation of *O-iso*-propylidene units while the effects on carbons bearing secondary hydroxyl functions seem to be less distinct.

The formation of **10** proved a *cis*-relationship between the OH groups at C-5 and C-6, and consequently both are β-orientated. The fact that no 6,7-*iso*-propylidene



9 R = H
11 R = Ac



10 R = H
12 R = Ac

derivative was formed [10], combined with the ^{13}C NMR data, confirms the *trans*-relationship between the 6-OH and 7-OH groups and thus the α-orientation of the latter.

The present results show the value of ^{13}C NMR spectroscopy as an aid to solve configurational problems in the iridoid glucosides. The structure and configuration now demonstrated for cynanchoside (**2**, 7α,10-dihydroxyharpagide) is suggested to arise from **1** by cleavage of the epoxide ring.

EXPERIMENTAL

CC was on Si gel, 70–230 mesh (Merck) and cellulose CF 11 (Whatman). Si gel SIF_{254} (Erba) and cellulose (Merck) plates were used for TLC. Paper chromatograms (PC) 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 3,5-dinitrosalicylic acid (3,5-dinitrosalicylic acid 0.5 g, NaOH 4 g, H_2O 100 ml), heating at 100° (cellulose plates and PC).

Isolation of iridoid fraction. Macfadyena cynanchoides was harvested in autumn in the Botanical Garden, University of Rome. A reference specimen (A5-1) has been deposited at the Botanical Institute Herbarium, University of Rome. Fresh aerial part (4 kg) was extracted twice with 90% EtOH (10 l. each) at room temp. for 3 days. PC (*n*-BuOH–HOAc–H_2O, 63:10:27) visualized with vanillin, showed 6 spots with the following R_f values: 0.42 (*A*, brown), 0.33 (*B*, pink) 0.29 (*C*, pink), 0.16 (*D*, grey-violet), 0.11 (macfadyenoside (**1**), brown), 0.05 (cynanchoside (**2**), brown). The ethanolic extracts were concd to an aq. suspension which was continuously extracted with petrol, bp 40–70° (2 l.). Decolorizing charcoal (1 kg) was added and the aq. suspension stratified on a Gooch funnel (20 cm dia) containing a layer of Si gel (100 g). Mono- and di-saccharides were removed with H_2O (12 l.) and 5% EtOH (8 l.). Compounds **1** and **2** (fraction I, 5 g) with 10% EtOH(8 l.); **1**, **2**, *C* and *D* (fraction II, 3 g) with 20% EtOH (7 l.) and finally a mixture of **1**, *A*, *B*, *C* and *D* with 50% EtOH (5 l.) (fraction III, 2.5 g) and 80% EtOH (4 l.) (fraction IV, 0.5 g).

Isolation of cynanchoside (**2**). Fractions I and II (8 g), chromatographed on cellulose (250 g) in *n*-BuOH satd with H_2O afforded the following fractions: (a) *C* and *D* (0.5 g), (b) **1** (3 g), (c) **1** and **2** (1 g). Fraction (c) was rechromatographed on Si gel eluted with Me_2CO–H_2O (9:1) and then on Si gel eluted with EtOAc–MeOH (7:3), to give pure **2** (400 mg) as an amorphous powder; $[\alpha]_D^{25}$ −126° (MeOH; *c* 1.0); UV λ_{max}^{MeOH} nm (log ε): 204 (3.6); IR ν_{max}^{KBr} cm^{-1}: 3400, 2940, 1670, 1075, 1025. (Found: C, 45.28; H, 6.25. $C_{15}H_{24}O_{12}$ requires: C, 45.45; H, 6.10%).

Heptaacetate (**5**) *and octaacetate* (**6**) *from* **2**. Compound **2** (100 mg) dissolved in dry pyridine (0.3 ml) was treated with Ac_2O (0.6 ml) for 1 hr at room temp. MeOH was added and after 20 min the soln was evapd and the residue, chromatographed on Si gel in Et_2O–EtOAc (19:1), gave the heptaacetate **5** (60 mg) as an amorphous powder. (Found: C, 50.20; H, 5.67. $C_{29}H_{38}O_{19}$ requires: C, 50.43; H, 5.55%). Compound **5** (60 mg), acetylated for 3 hr as described above for **2**, gave a residue which was chromatographed on Si gel in Et_2O–EtOAc (19:1) to give the octaacetate **6** (40 mg) as an amorphous powder. $[\alpha]_D^{25}$ −141° (MeOH; *c* 0.7). (Found: C, 50.63; H, 5.63. $C_{31}H_{40}O_{20}$ requires: C, 50.82; H, 5.50%).

8,10-O-Iso-*propylidene cynanchoside* (**9**) *and* 5,6-8,10-*bis*-O-iso-*propylidenecynanchoside* (**10**). Compound **2** (260 mg) was treated with a 15% soln of $SnCl_2$ in dry Me_2CO (4 ml) adding acetonedimethylketal (0.1 ml). The suspension was stirred at room temp. for 1.5 hr then poured into cold, satd $NaHCO_3$ soln. The resulting suspension was centrifuged and the residue washed twice with Me_2CO–H_2O (1:1, 10 ml). The collected solns showed on TLC (*n*-BuOH–MeOH–H_2O, 7:1:3) the presence of two major compounds with higher R_f than **2** and were evapd to dryness to give an amorphous residue which, chromatographed on Si gel with *n*-BuOH satd with H_2O, gave **10** (56 mg) and **9** (42 mg) as amorphous powders.

Hexaacetate (**11**) *from* **9**. Compound **9** (42 mg) was treated with pyridine (0.5 ml) and Ac_2O (1 ml) for 2 hr at room temp. The mixture, worked up as previously described for **5**, afforded an amorphous residue which, on TLC in Et_2O, showed only the presence of **11**. The residue chromatographed on Si gel in Et_2O gave **11** (28 mg) as an amorphous powder. (Found: C, 52.09; H, 5.99. $C_{30}H_{40}O_{18}$ requires: C, 52.32; H, 5.86%).

Pentaacetate **12** *from* **10**. Compound **10** (56 mg), acetylated as described for **5**, gave an amorphous residue which on TLC in Et_2O–hexane (4:1) showed only the presence of **12**. The residue chromatographed on Si gel in Et_2O–hexane (4:1) afforded **12** (30 mg) as an amorphous powder. (Found: C, 53.98; H, 6.26. $C_{31}H_{42}O_{17}$ requires: C, 54.22; H, 6.17%).

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