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IRIDOID GLUCOSIDES FROM *UTRICULARIA AUSTRALIS* AND *PINGUICULA VULGARIS* (LENTIBULARIACEAE)

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Key Word Index—*Utricularia australis*; *Pinguicula vulgaris*; Lentibulariaceae; iridoid glucosides; aucubin; gardoside; mussaenosidic acid; 6-deoxycatalpol; catalpol; globularin; 10-(*Z*)-cinnamoyl-catalpol; taxonomy.

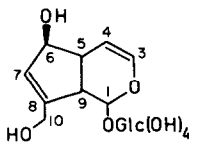
Abstract—*Utricularia australis* contained 6-deoxycatalpol, a new iridoid glucoside, besides aucubin, gardoside and mussaenosidic acid. From *Pinguicula vulgaris* was isolated catalpol, globularin and 10-(*Z*)-cinnamoyl-catalpol, the latter being a new compound. Thus, the iridoids found in Lentibulariaceae belong to structural types which are common in Scrophulariaceae and related families.

INTRODUCTION

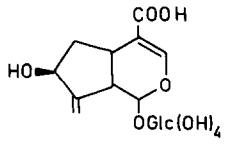
For some time members of the small insectivorous family Lentibulariaceae have been known to contain iridoid glucosides. Thus, by chromatographic methods, aucubin (**1**) has been detected in *Utricularia* [cf. 1] and catalpol (**7**) in *Pinguicula* [2]. We have performed a more detailed investigation of *Utricularia australis* R. Br. and *Pinguicula vulgaris* L. and shown that both species contain several iridoid glucosides.

RESULTS AND DISCUSSION

From *U. australis* the known iridoid glucosides aucubin (**1**), gardoside (**2**) and mussaenosidic acid (**3**) were isolated in addition to the new 6-deoxycatalpol (**4**). The ^{13}C NMR spectrum (Table 1) of **4** exhibited 15 signals, six of which belonged to a β-glucopyranosyl moiety. The remaining signals appeared to be best accommodated by structure **4**. Thus the signals at δ62.4 (*d*) with a large coupling constant [3] (J = 186 Hz), and 69.7 (*s*) showed the presence of a 7,8-epoxy grouping. The ^1H NMR spectrum (see Experimental) was also in accordance with structure **4**. Assuming the usual stereochemistry at C-1, C-5 and C-9, only that of the epoxy group is left undecided. The known dependence of the substitution pattern of the iridoid moiety upon the position of the C-9 NMR shift [4–6] makes it possible to calculate the C-9 shift for **4**. Using catalpol (**7**) as the model and subtracting the increment for the 6β-OH substituent (−1.7 ppm [6]) the expected value for the C-9 shift is δ44.2, comparing favourably with the value found (43.2). (Although the increment for a 7α,8α-epoxy grouping is unknown, 8-epimeric compounds with an 8,10-dioxygen substitution pattern usually differ by *ca* 5 ppm in their C-9 shift values [6].) In order to prove the position of the epoxy group, **4** was subjected to the known [7] base catalysed ring opening reaction in which the 10-hydroxy group exerts a neighbouring group effect causing inversion at the C-8 centre. The structure of the major product (**5**) from the reaction was readily deduced from the ^{13}C NMR spectrum of **5a**. Firstly, the magnitude of the C-9 shift (δ44.4) proves the 8α-OH stereochemistry



1



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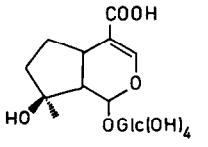

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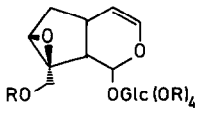
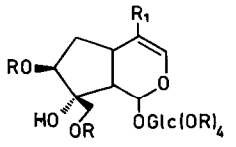
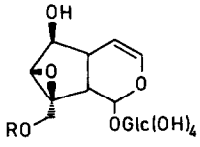


4 R = H
4a R = Ac





5 R = R_1 = H
5a R = Ac; R_1 = H
6a R = Ac; R_1 = COOMe

7 R = H
8 R = *Z*-cinnamoyl
9 R = *E*-cinnamoyl

[8]; secondly, the C-7 and C-8 shifts (δ81.6 and 79.8) prove the *trans*-disposition of the hydroxyl groups at these carbon atoms. In fact, when the spectrum of **5a** is compared with that of **6a** [8] all corresponding shifts (except C-3 and C-4) fall within 1.4 ppm, thus establishing the identical substitution patterns on the cyclopentane rings. In view of the expected inversion at C-8 in the ring-

Table 1. ^{13}C NMR data

C	4	4a	5	5a	7	8	9	Methyl cinnamate [9] (Z)	(E)
1	95.3 (167)	94.4 (164)	94.7	92.7	95.5	95.0	94.7		
3	140.3 (191)	140.3 (189)	139.1	139.1	141.3	141.0	140.9		
4	106.7 (165x)	105.3 (163)	109.4	106.7	104.0	103.3	103.2		
5	31.6 (140x)	31.1 (137)	28.3	28.4	38.1	37.5	37.5		
6	35.0 (130x)	35.3 (130x)	38.3	35.7	78.4	77.9	77.9		
7	62.4 (186)	59.8 (184)	83.3	81.6	62.9	62.6	62.0		
8	69.7 s	65.0 s	79.3	79.8	66.6	63.4	62.9		
9	43.2 (138x)	42.6 (138)	44.6	44.4	42.5	42.1	42.3		
10	61.5 (146)	63.9 (149)	65.6	66.8	60.9	64.0	63.6		
1′	99.5 (161x)	96.9 (164)	99.0	96.0	99.4	99.0	99.0		
2′	73.7	71.0	73.5	70.4	73.6	73.4	73.5		
3′	76.5	72.5	76.5	71.8	76.4	76.2	76.2		
4′	70.4	68.7	70.4	68.1	70.3	70.1	70.0		
5′	77.0	73.0	77.0	72.1	77.0	76.7	76.7		
6′	61.5	61.5	61.5	61.5	61.5	61.5	61.5		
C=O						168.6	168.0	166.4	168.5
α						119.3	117.3	119.2	119.1
β						145.4	146.2	143.2	146.0
1″						135.5	134.2	134.7	135.4
2″						129.6	128.6	129.7	129.3
3″						128.9	129.7	127.9	130.1
4″						129.2	131.1	129.0	131.5

The spectra were recorded in D_2O (glucosides) or in $CDCl_3$ (esters) at 22.6 MHz, except for **5** (67.9 MHz). All spectra were aligned to C-6′ = δ61.5 [6].

opening reaction, the structure of the new compound must be the one shown in the figure (**4**).

Pinguicula vulgaris contained the known iridoid glucosides catalpol (**7**) and globularin (**9**) besides a small quantity of a new iridoid identified as 10-(*Z*)-cinnamoylcatalpol (**8**). The structure of the latter was readily deduced by NMR spectroscopy. In the ^1H NMR spectrum of **8** the shifts and coupling constants (δ7.01 and 5.99; $J = 13$ Hz) arising from the β- and α-protons of the cinnamoyl group were very different from those observed in the spectrum of **9** (δ7.71 and 6.56; $J = 16$ Hz) and demonstrated the presence of a (*Z*)-cinnamoyl moiety in **8**. This was confirmed by comparison with the ^{13}C NMR spectra of methyl (*Z*)-and (*E*)-cinnamate [9] (Table 1). Furthermore, the site of esterification was established as C-10 by comparison with the spectrum of globularin (**9**) and by finding the same acetylation shifts for C-8 and C-10 in the catalpol moieties of **8** and **9**. Since **8** was isolated in a very small amount and since neither **8**, nor its pentaacetate (**8a**), could be obtained crystalline, no further attempts to characterize the compound were made.

The present work demonstrates that members of the Lentibulariaceae contain iridoids of types which are common in Scrophulariaceae and related families [1, 10], supporting the view that Lentibulariaceae most probably is a true member of the order Scrophulariales.

EXPERIMENTAL

Microanalyses were carried out at the Novo Microanalytical Laboratory, Bagsværd, Denmark. Mps are corrected. *Utricularia australis* R. Br. (IOK-8/82) was collected at Uggerby Strand, Denmark, and *Pinguicula vulgaris* L. (IOK-54/79) at Børsmose, Henne, Denmark and at Öland, Sweden. Vouchers have been deposited at the Botanical Museum, Copenhagen; they were identified by Dr. Alfred Hansen.

U. australis. Whole plants (4.2 kg, wet wt) were homogenized in EtOH (*ca* 12 l), and the extract taken to dryness and partitioned in H_2O–Et_2O. The aq. fraction was passed through wet Al_2O_3 (400 g) followed by H_2O (1 l). Evaporation gave a coloured residue which was dissolved in 85% MeOH and passed through a mixture of charcoal (100 g) and celite (50 g) and washed with MeOH (1 l). Evaporation gave a colourless product (13.4 g) which was applied to a home-made low-pressure reversed-phase

column loaded with 2 kg of silica gel-60, 40–63 μ [11]. Elution was done with H_2O–MeOH (32 ml/min; 10:1) and monitored by UV at 254 and 206 nm. Carbohydrates were first eluted, followed by a fraction adsorbing at 254 nm (*Fract. A*, 1.16 g). The next fraction was *aucubin* (**1**, 0.62 g; 0.014%) identified by ^{1}H NMR, followed by *6-deoxycatalpol* (**4**, 0.45 g; 0.01%), crystallized from EtOH, mp 212.5–214°; $[\alpha]_D^{22}$ −47° (MeOH; *c* 0.4); ^{1}H NMR (90 MHz, D_2O): δ6.33 (*dd*, *J* = 6 and 1 Hz; H-3), 5.04 (*m*, 2H, H-1 and H-4), 4.83 (*d*, *J* = 7 Hz, H-1'), 4.35 and 3.81 (*AB*, *J* = 13 Hz, 10-CH_2), 3.65 (*br s*, H-7), 2.4 (*m*, 3H, H-5, H-6 and H-9), 1.6 (*m*, H-6). (Found: C, 51.8; H, 6.5. $C_{15}H_{22}O_9$ requires: C, 52.0, H, 6.4%.)

6-Deoxycatalpol pentaacetate (**4a**) was crystallized from EtOH, mp 133–134.5°; $[\alpha]_D^{24}$ −16° ($CHCl_3$, *c* 1.2); ^{1}H NMR (400 MHz, $CDCl_3$): δ6.27 (*dd*, *J* = 6 and 2.5 Hz, H-3), 4.93 (*dd*, *J* = 4 and 6 Hz, H-4), 4.89 and 3.92 (*AB*, *J* = 13 Hz, 10-CH_2), 4.79 (*d*, *J* = 9 Hz, H-1), 3.46 (*br s*, H-7), 2.5 (2H, H-5 and H-9), 2.35 (*dd*, *J* = 14 and 7 Hz, H-6), 1.44 (*dd*, *J* = 14 and 9 Hz, H-6). (Found: C, 53.9; H, 5.8. $C_{25}H_{32}O_{14}$ requires: C, 54.0, H, 5.8%.)

Fraction A from above was mixed with HOAc (1 ml) and the product was rechromatographed on the same column using H_2O–MeOH (5:1) as the eluent. Following the carbohydrate fraction came *gardoside* (**2**, 13 mg, 0.0003%) and then *mussaenosidic acid* (**3**, 220 mg, 0.005%) both of which were identified by the ^{1}H NMR spectra [11].

Ring opening of **4** [7]. A soln of **4** (125 mg) in satd $Ba(OH)_2$ soln (5 ml) was heated to 70° for 4 hr. After neutralization with HOAc two compounds (*ca* 4:1) were detected (HPLC). The mixture was applied to a preparative column (Merck Lobar RP-8 (B)) and eluted with H_2O–MeOH (10:1). After several runs two fractions were obtained. From the first and major fraction **5** (40 mg) was obtained as a glass and characterized by ^{13}C NMR (Table 1) and ^{1}H NMR (90 MHz, D_2O): δ6.26 (*dd*, *J* = 2 and 6.5 Hz, H-3), 5.44 (*d*, *J* = 2.5 Hz, H-1), 5.07 (*dd*, *J* = 3 and 6.5 Hz, H-4), 4.17 (*t*, *J* = 5.5 Hz, H-7), 3.78 and 3.73 (*AB*, *J* = 13 Hz, 10-CH_2), 2.92 (*m*, H-5), 2.43 (*dd*, *J* = 2.5 and 9.5 Hz, H-9), 1.94 (*dd*, *J* = 5.5 and 6.5 Hz, 6-CH_2). Acetylation provided the hexaacetate (**5a**) as a syrup; $[\alpha]_D^{21}$ −92° ($CHCl_3$, *c* 1.3); ^{13}C NMR (Table 1); ^{1}H NMR (90 MHz, $CDCl_3$): δ6.22 (*dd*, *J* = 2 and 6 Hz, H-3), 5.35 (*d*, *J* = 3 Hz, H-1), 5.08 (*t*, *J* = *ca* 6 Hz, H-7), 4.88 (*dd*, *J* = 2.5 and 6 Hz, H-4), 4.21 and 4.04 (*AB*, *J* = 12 Hz, 10-CH_2) 3.22 (*s*, OH), 2.74 (*m*, H-5), 2.43 (*dd*, *J* = 3 and 9 Hz, H-9), 2.09–2.00 (6 × OAc), 1.93 (*t*, *J* = 6 Hz, 6-CH_2). (Found: C, 52.4; H, 6.1. $C_{27}H_{36}O_{16}$ requires: C, 52.6; H, 5.9%.)

P. vulgaris. Whole plants (17 g) were worked up as above. The glycosidic fraction was separated using reversed-phase chromatography [Merck Lobar RP-8 (C)] with H_2O–MeOH (10:1 to 1:1). Three iridoid-containing fractions were obtained: (i) *Catalpol* (**7**, 72 mg, 0.4%) identified by its NMR spectra. (ii) *10-(Z)-cinnamoyl-catalpol* (**8**, 11 mg, 0.06%); due to the small amount available this compound was characterized solely by ^{13}C NMR (Table 1) and by ^{1}H NMR (90 MHz, Me_2CO-d_6): δ7.71–7.29 (arom. H), 7.01 (*d*, *J* = 13 Hz, β-H), 6.33 (*dd*, *J* = 6 Hz and 1.5 Hz, H-3), 5.99 (*d*, *J* = 13 Hz, α-H), 5.04 (*m*, H-4), 5.00 (*d*, *J* = 9 Hz, H-1), 4.92 and 4.21 (*AB*, *J* = 13 Hz, 10-CH_2), 4.74 (*d*, *J* = 7 Hz, H-1'), 3.90 (*br d*, *J* = 7 Hz, H-6), 3.34 (*br s*, H-7), 2.44 (*dd*, *J* = 9 and 8 Hz, H-9), 2.25 (*m*, H-5). The penta-acetate could not be purified completely by prep. TLC and appeared non-crystalline. Due to the small amounts available, further characterization was abandoned. (iii) *Globularin* (**9**, 62 mg, 0.4%) ^{13}C NMR (Table 1); ^{1}H NMR (90 MHz, Me_2CO-d_6): δ7.73–7.38 (arom. H), 7.71 and 6.56 (*AB*, *J* = 16 Hz, H-β and H-α), the remaining signals virtually as for **8**. Pentaacetate: mp (EtOH) 151–152°; $[\alpha]_D^{18}$ −97° ($CHCl_3$, *c* 0.9). Lit. [12]: mp 147–149°; $[\alpha]_{578}^{20}$ −103° ($CHCl_3$, *c* 1.0).

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