236. Iridoid and Aryl Glucosides from *Globularia nudicaulis* and *Globularia nana*[1])

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Summary

The iridoid and aryl glycosidic constituents of *Globularia nudicaulis* L. and *G. nana* LAM. are reported. The structure determination of lytanthosalin (10-*O*-(*E*)-cinnamoylaucubin), a new acylated iridoid glucoside, isolated from the latter species, is described.

Introduction. – We recently reinvestigated in detail the glycosides from the genus *Globularia*, using modern techniques, and isolated several new iridoid glucosides [1–4]. We have now examined the glycosidic constituents of *Globularia nudicaulis* L. and *G. nana* LAM. No work has yet been reported on the glycosidic constituents of *G. nana* while previous investigations on *G. nudicaulis* are limited to the identification (PC.) of asperuloside, aucubin and monotropein [5] [6].

Results and discussion. – From the aerial parts of *G. nudicaulis*, three major iridoids, asperuloside (**1**), aucubin (**2**) and melampyroside (**3**) and the glucosides A, (**7**) and B, (**8**) were isolated by fractionation of the methanolic extract through a silica gel column followed by preparative and semi-preparative HPLC. on a reversed phase C_{18}-column. However, we failed to detect the presence of monotropein in this plant.

The identity of the iridoid glucosides was established by chromatography (HPLC., TLC.) and from the spectral data. The β-D-configuration at C(1′) in the aryl glucoside B (**8**) came from the coupling constant ($J_{1',2'}$ = 8 Hz) in the ^{1}H-NMR. and from the chemical shift value of C(1′) (96.16 ppm) in the ^{13}C-NMR. [7]. The site of benzoylation at C(3′) in **8** was determined by comparing the spectra with that of the β-D-glucose [7]. Likewise, the α-D-configuration at C(1′) in the aryl glucoside A (**7**) was established from its NMR. spectral data [^{1}H-NMR.: $J_{1',2'}$ = 4 Hz; ^{13}C-NMR.: 91.31 ppm, C(1′)] and the site of benzoylation at C(2′) by comparing the ^{13}C-NMR. spectral data with that of the α-D-glucose [7]. To the best of our knowledge the two aryl glucosides **7** and **8** have not been encountered before in nature.

[1]) Part 5 in the series 'Glycosides of Globulariaceae'. Part 4: [4].

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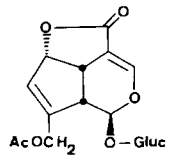
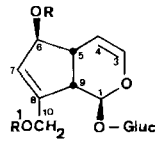
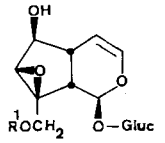


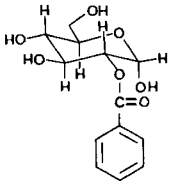
1 Asperuloside



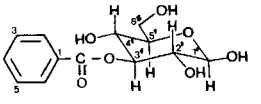
2 Aucubin
R = R[1] = H
3 Melampyroside
R = H; R[1] = benzoyl
4 Lytanthosalin
R = H; R[1] = (*E*)-cinnamoyl
4a Lytanthosalinpentaacetate
R = Ac; R[1] = (*E*)-cinnamoyl



5 Catalpol
R = R[1] = H
6 Globularin
R = H; R[1] = (*E*)-cinnamoyl



7 2′-Benzoyl-*α*-D-glucoside



8 3′-Benzoyl-*β*-D-glucoside

Likewise, the aerial parts of *G. nana* afforded four known iridoids, asperuloside (**1**), aucubin (**2**), catalpol (**5**) and globularin (**6**), together with the new acyl iridoid **4**, the structure of which was based upon the following evidence.

The ^{1}H- and ^{13}C-NMR. spectra of melampyroside (**3**) [4] and **4** were similar, except that **4** showed additional signals. Two extra peaks were present in the ^{13}C-NMR. spectrum at 146.60 and 118.42 ppm and an *AB*-system was observed

Table 1. *^{13}C-NMR. spectral data* (CD_3OD) *of aucubin* (**2**), *melampyroside* (**3**) *and lytanthosalin* (**4**)

C-Atom	2	3	4	C-Atom	2	3	4
C(1)	97.72	97.83	97.89	C(1″)	–	131.02	135.42
C(3)	141.49	141.64	141.58	C(2″)/(6″)	–	130.54[b])	129.91[b])
C(4)	105.72	105.51	105.48	C(3″)/(5″)	–	129.59[b])	129.21[b])
C(5)	46.07	46.04	46.08	C(4″)	–	134.39	131.51
C(6)	82.74	82.66	82.65				
C(7)	130.27	132.59	132.46	C(*α*)	–	–	146.60
C(8)	147.95	142.41	142.35	C(*β*)	–	–	118.42
C(9)	47.89	48.39	48.24	CO	–	167.71	168.14
C(10)	61.32	63.98	63.50				
C(1′)	99.90	100.08	100.07				
C(2′)	74.81	74.72	74.67				
C(3′)	78.07[a])	77.99[a])	77.94[a])				
C(4′)	71.44	71.27	71.25				
C(5′)	77.77[a])	77.72[a])	77.67[a])				
C(6′)	62.60	62.61	62.61				

[a])[b]) Values with same superscript in the vertical column are interchangeable.

Table 2. *^{13}C-NMR. spectral data* (CD_3OD) *of 2′-benzoyl-α-D-glucoside* **7** *and 3′-benzoyl-β-D-glucoside* **8**

Compound	C(1)	C(2,6)	C(3,5)	C(4)	CO	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	C(6′)
7	130.83	130.72[a])	129.46[a])	134.19	167.76	91.31	76.04	72.22	71.81	72.93	62.63
8	130.48	130.84[b])	129.58[b])	134.76	166.83	96.16	73.84	78.65	70.87	77.83	62.16

[a])[b]) Values with the same superscript in the same line are interchangeable.

in the ^{1}H-NMR. spectrum at 6.54 and 7.70 ppm ($J = 16$ Hz). The assignment for the ^{13}C-NMR. signals *(Table 1)* were made with the aid of our results on related iridoids [8]. Hydrolysis of **4** with methanolic NaOH-solution afforded (*E*)-cinnamic acid and aucubin (**2**), identified with authentic samples. Comparison of the ^{13}C-NMR. spectra of **4** and **2** revealed the site of acylation at HO–C(10) [8]. The above mentioned chemical and spectral data show that the acyl iridoid glucoside **4** is 10-*O*-((*E*)-cinnamoyl)aucubin. An acyl iridoid named lytanthosalin, was isolated from *Lytanthus salicinus* (LAM.) Wettst. (= *Globularia salicina* LAM.) by *Fikenscher et al.* [9][3]). The nature of the compound was established to be a ((*E*)-cinnamoyl)-aucubin but the site of acylation was left unassigned. We speculated that this compound might be identical with that of the compound **4** of *G. nana*, based on the fact that all the acyl iridoids of Globulariaceae isolated so far have an acylated HO–C(10) function. A direct comparison of **4** with that of lytanthosalin proved the identity of the two compounds and supported our taxonomic speculation.

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Experimental Part

General. General procedures are the same as reported [3] [4]. Spectral data: UV. [λ_{max} nm ($\log\varepsilon$)], IR. (cm^{-1}), ^{1}H- and ^{13}C-NMR. (δ in ppm, J in Hz).

Extraction and purification of iridoids from G. nudicaulis. Fresh above-ground parts of *G. nudicaulis* (1.8 kg) collected from the Klausenpass region, Switzerland, in May 1978, were cut into small pieces and extracted with petroleum ether (3×3 l) and then with MeOH (3×3 l) at 40°. The MeOH extract was concentrated to dryness and the residue was taken up in H_2O. The water-soluble portion was passed through a prewashed (H_2O) column of neutral Al_2O_3 (500 g), eluting with H_2O. The eluate was concentrated and lyophilized to give the crude glycoside fraction (117.9 g). A portion of the mixture (30 g) was chromatographed over silica gel (400 g, 65×4.3 cm), in CH_2Cl_2/MeOH/H_2O [80:20:2 (3 l), 70:30:3 (1.5 l), 60:40:4 (2 l)]. Four major fractions, A (12.65 g), B (2.63 g), C (1.6 g) and D (7.39 g) were collected.

Fr. A (2 g), which was subjected to preparative LC. [reversed phase column; MeOH/H_2O 45:55; flow rate 100 ml/min], gave 3 major fractions A1, A2 and A3.

Asperuloside (**1**). Fr. A1 on usual processing gave an amorphous powder (0.38 g), crystallized from H_2O as needles, m.p. 129–131°. The Rf, UV., ^{1}H- and ^{13}C-NMR. data were identical with that of asperuloside.

[3]) The same authors [9] have isolated another acyl iridoid, named rhinanthoglabrin, from *Rhinanthus glaber* LAM. (= *Rh. crista-galli* L.P.P. = *Rh. major* EHRH.). Rhinanthoglabrin was proved to be a benzoylaucubin with unknown site of acylation. TLC. and HPLC. analyses in our laboratory showed the identity of rhinanthoglabrin with melampyroside. The name melampyroside for 10-*O*-benzoylaucubin has thus priority over rhinanthoglabrin.

2'-Benzoyl-α- (**7**) *and 3'-benzoyl-β-*D*-glucosides* (**8**). Fr. A2 (0.2 g) was further subjected to semi-preparative scale reversed phase HPLC. [*μBondapak* C_{18} column; 30 cm × 8 mm I.D.; MeOH/H_2O 10:90; flow rate 4 ml/min] to give 3 fractions A2X (0.03 g), A2Y (0.05 g) and A2Z (0.04 g).

*2'-Benzoyl-α-*D*-glucoside* (7). By spectral analysis Fr. A2X was shown to contain a mixture of 2'-benzoyl-α- and 3'-benzoyl-β-D-glucosides (see below). – ^{1}H-NMR. (CD_3OD): 5.40 (*d*, $J_{1',2'}$ = 4, 1H, H–C(1')). – ^{13}C-NMR. (CD_3OD): see *Table 2*.

*3'-Benzoyl-β-*D*-glucoside* (**8**). Fr. A2Y on usual work-up gave an amorphous powder, $[\alpha]_D^{25} = -13.1°$ (c = 0.75, MeOH). – IR. (KBr): 3410, 3300, 1715, 1600, 1585, 1450. – ^{1}H-NMR. (CD_3OD): 8.0–8.14 (2 arom. H); 7.36–7.66 (3 arom. H); 5.74 (*d*, $J_{1',2'}$ = 8, 1H, H–C(1')). – ^{13}C-NMR. (CD_3OD): see *Table 2*.

Fr. A2Z gave a further crop of asperuloside (**1**).

Melampyroside (**3**). Fr. A3 on usual work-up gave an amorphous powder (1.1 g) identical with authentic melampyroside.

Fr. B contained (HPLC.) asperuloside (**1**), 2'-benzoyl-α- (**7**) and 3'-benzoyl-β-D-glucosides (**8**).

Aucubin (**2**) was isolated (0.8 g) from Fr. C by prep. LC. [reversed phase column; MeOH/H_2O 10:90; flow rate 100 ml/min] and identified as usual.

Fr. D. contained mainly sugars and was not further examined.

Extraction and purification of iridoids from G. nana. Dried and milled above-ground parts (20 g) of *G. nana*, collected in Provence, France, in July 1979, were processed as described for *G. nudicaulis* to give an amorphous pale yellow solid (1.47 g) which was subjected to low pressure LC. over a reversed phase C_{18} column (particle size: 50 μm; 15 mm, I.D.) using MeOH/H_2O [30:70 (2 l) and 50:50 (0.5 l)] and 4 major fractions A (0.69 g), B (0.22 g), C (0.28 g) and D (0.12 g) were collected. Aucubin (**2**) and catalpol (**5**) in Fr. A and asperuloside (**1**) and globularin (**6**), respectively in Fr. B and C were detected by TLC. and HPLC.

*Lytanthosalin (10-O-((*E*)-cinnamoyl)aucubin* (**4**). Fr. D (0.12 g) was further purified by a silica gel column using CH_2Cl_2/MeOH/H_2O 80:20:2 to give pure **4** (0.08 g) as an amorphous powder, $[\alpha]_D^{20} = -84.44°$ (c = 0.55, MeOH). – UV. (MeOH): 216 (4.10), 222 *S*, 278 (4.42). – IR. (KBr): 3400, 1710, 1655 *S*, 1635, 1580, 1495, 1450. – ^{1}H-NMR. (CD_3OD): 7.72 (*d*, J(gem) = 16, H–C(α)); 7.32–7.62 (5 arom. H); 6.54 (*d*, J(gem) = 16, H–C(β)); 6.33 (*d* × *d*, J(3,5) = 2.0, J(3,4) = 6.5, H–C(3)); 5.81 (*t* × *d* × *d*, J(6,7) = 1.5, J(7,9) = 1.0, H–C(7)); 5.11 (*d* × *d*, J(4,5) = 4.0, J(3,5) = 6.0, H–C(4)); 4.95 (*d*, J(1,9) = 7.0, H–C(1)); 4.69 (*d*, J(1',2') = 7, H–C(1')); 4.94 (*m*, 2H–C(10)); 4.38–4.54 (*m*, H–C(6)); 2.96 (*d* × *d* × *d*, J(5,9) = 8, J(1,9) = 7, J(7,9) = 1, H–C(9)); 2.58–2.78 (*m*, H–C(5)). – ^{13}C-NMR. (CD_3OD): see *Table 1*.

*10-O-((*E*)-Cinnamoyl)aucubin pentaacetate* (**4a**). Acetylation with acetic anhydride and pyridine at RT. gave after work-up an amorphous powder, $[\alpha]_D^{25} = -124.3°$ (c = 0.58, $CHCl_3$). – IR. (KBr): 1750, 1720, 1665, 1640, 1580, 1500, 1450. – ^{1}H-NMR. ($CDCl_3$): 7.70 (*d*, J(gem) = 16, H–C(α)); 7.30–7.60 (5 arom. H); 6.44 (*d*, J(gem) = 16, H–C(β)); 6.16 (*d* × *d*, J(3,5) = 2.0, J(3,4) = 6.5, H–C(3)); 5.88 (*t* × *d* × *d*, J(6,7) = 1.5, J(7,9) = 1.0, H–C(7)); 4.78–5.36 (*m*, H–C(1), H–C(6), H–C(4), 2H–C(10), H–C(1'), H–C(2'), H–C(3') and H–C(4')); 4.10–4.24 (*m*, 2H–C(6')); 3.70–3.90 (*m*, H–C(5')); 3.14–3.32 (*m*, H–C(9)); 2.74–2.92 (*m*, H–C(5)); 1.96–2.08 (5 × CH_3COO). – MS.: 686 (< 1, M^+), 627 (2.5, $M^+ - 59$), 339 (< 1, $M^+ - 347$), 279 (12.5, $M^+ - (347 + 60)$), 131 (62, $M^+ - (347 + 60 + 17 + 131)$); fragments due to the glucose part: 331 (75), 271 (25), 211 (13), 169 (100), 109 (55), 43 (50); fragments due to the cinnamoyl part: 131 (62), 103 (15), 77 (7).

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