0°, with stirring, under Ar The soln was stirred at room temp. for 15 hr, and then made acidic with excess AcOH and concd under red pres to leave a syrup, which was partitioned between EtOAc and H_2O The EtOAc soln was washed with aq satd NaCl and then dried (Na_2SO_4) After filtration, the filtrate was concd under red pres and then purified by prep TLC (Kieselgel 60 F_{254}, 0 5 mm) using hexane–EtOAc (1 1) to give orange needles of plumbagin (3, 3 5 mg) (mp, TLC and IR spectrum)

Conversion of diomuscipulone (2) *to triacetates* 5 *and* 6 To a soln of 2 (5 mg) in THF (2 ml) was added $LiAlH_4$ (10 mg), with stirring, at 0°, and then the reaction soln was further stirred at room temp for 15 hr After acidification with 2 N HCl, the reaction mixture was diluted with H_2O and extracted with EtOAc The EtOAc soln was washed with aq satd NaCl, and then dried (Na_2SO_4) After filtration, the filtrate was concd under red pres to give an oil, which was dissolved in Ac_2O–pyridine (1 1, 1 ml) and allowed to stand at room temp for 15 hr The reaction soln was concd under red pres to give an oily residue, which was separated by repeated prep TLC (Kieselgel 60 F_{254}, 0 5 mm) using hexane–EtOAc (1 1) and then $CHCl_3$–EtOAc (9 1) to afford 5 (2 7 mg) and 6 (1 2 mg) Compound 5, colourless oil IR ν_{max}^{film} cm^{-1} 1775, 1740, 1620, 1600, 1485, ^1H NMR ($CDCl_3$) δ1 46 (3H, *s*), 2 00 (3H, *s*), 2 07 (3H, *s*), 2 1–1 95 (2H, overlapped with OAc signals), 2 30 (3H, *s*), 4 20 (2H, *t* (*br*), J = 7 Hz), 6 10 (1H, *s*), 6 9–7 3 (3H, complex, overlapped with the solvent signal), MS *m/z* (rel int) 336 $[M]^+$ (90), 294 (100), 277 (36), 252 (33), 250 (93), 234 (74), 217 (45), 208 (29), MS *m/z* 336 1204 $[M]^+$, calc for $C_{17}H_{20}O_7$ *m/z* 336 1208 Compound 6, colourless oil IR ν_{max}^{film} cm^{-1} 1775, 1740, 1610, 1600, 1485, ^1H NMR ($CDCl_3$) δ1 40 (3H, *s*), 2 03 (3H, *s*), 2 07 (3H, *s*), 2 28 (3H, *s*), 2 0–2 3 (2H, overlapped with OAc signals), 4 29 (2H, *t*, J = 7 Hz), 5 97 (1H, *s*), 6 9–7 3 (3H, complex, overlapped with the solvent signal), MS *m/z* (rel int) 336 $[M]^+$ (23), 294 (100), 252 (8), 234 (7), 217 (8), MS *m/z* 336 1208 $[M]^+$ (calc for $C_{17}H_{20}O_7$ 336 1208)

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IRIDOID GLUCOSIDES FROM *MELAMPYRUM*

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Key Word Index—*Melampyrum arvense*, *M cristatum*, Scrophulariaceae, iridoid glucosides, gardoside methyl ester, mussaenosidic acid, aucubin, 8-epiloganin, mussaenoside, melampyroside

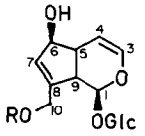
Abstract—*Melampyrum arvense* and *M cristatum* contain, besides aucubin, 8-epiloganin and melampyroside, a new natural iridoid glucoside gardoside methyl ester In addition, *M arvense* contains mussaenoside and *M cristatum* mussaenosidic acid, another novel iridoid glucoside

INTRODUCTION

Chromatographic investigations [1, 2] have shown that aucubin (1) and catalpol, as well as esters of these compounds, are common in the genus *Melampyrum* However, only two species have so far been investigated in detail From *M silvaticum* L, aucubin (1) and melampyroside (3) [3] and, more recently, mussaenoside (2), globularifolin, catalpol and monomelittoside were isolated [4] From *M laxum* Miq, 1, 2 and 3 have been obtained [5] In the present work we give details of the isolation and characterization of iridoid glucosides from *M arvense* L and *M cristatum* L

RESULTS AND DISCUSSION

Five iridoid glucosides were isolated from *M arvense*, namely aucubin (1), mussaenoside (2), melampyroside (3), 8-epiloganin (4) and gardoside methyl ester (5) Compounds 1–4 were identified by their ^1H and ^{13}C NMR spectra [6–8], while the structure of 5 was deduced in the following way Its ^{13}C NMR spectrum displayed signals corresponding to an iridoid glucoside substituted at C-4 with a methoxycarbonyl group Additional signals at δ151 2 (*s*) and 113 9 (*t*) proved the presence of an exocyclic double bond, other structural features included a carbon atom substituted with a



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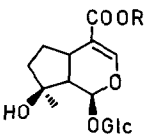
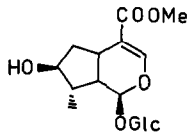
3 R = Bz



2 R = Me
6 R = H



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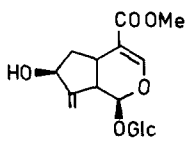
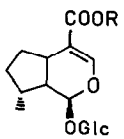


5



7 R = Me
8 R = H

hydroxyl group (δ 73 1, *d*) and a CH_2-group (δ 39 3, *t*) The ^{1}H NMR spectrum was in accordance with a structure such as **5** and acetylation of the glucoside gave a pentaacetate with melting point and specific rotation data close to those reported for gardoside methyl ester pentaacetate [9] Finally, hydrogenation of **5** over a Rh–C catalyst [10] provided a *ca* 9 1 mixture of **4** and loganin, establishing the stereochemistry at C-7 We were unable to confirm the earlier reported presence of catalpol in this plant [2]

M cristatum contained the same compounds as *M arvense*, with the exception that **2** had been replaced by mussaenosidic acid (**6**) The ^{1}H and ^{13}C NMR spectra of **6** and **2** (see ^{13}C NMR spectra in Table 1) were virtually identical, except for the resonance of the methoxy group (δ 52 6) in the latter As a proof, saponification of **2** gave a product indistinguishable from **6** (^{1}H NMR and HPLC)

A derivative of **6**, namely 2′-*p*-hydroxybenzoyl mussaenosidic acid is present in *Vitex negundo* (Verbenaceae) [11] Biosynthetically, it seems likely that all the iridoids in *Melampyrum* arise from 8-*epi*-deoxyloganic acid (**8**) We have shown earlier that 8-epiloganin (**4**) is biosynthesized from 8-*epi*-deoxyloganin (**7**) in *M cristatum* [12], and that 8-*epi*-deoxyloganic acid (**8**) is converted into aucubin (**1**) in several species within Scrophulariales [13, 14]

EXPERIMENTAL

Microanalyses Novo Microanalytical Laboratory, Bagsværd, Denmark *M arvense* (IOK-52/79) was collected in Åhus, Sweden, *M cristatum* (IOK-13/80) in Kongsøre Skov, Denmark Vouchers have been deposited at the Botanical Museum, Copenhagen, and were identified by Dr Alfred Hansen

Table 1 ^{13}C NMR data of iridoid glucosides from *M arvense* and *M cristatum**

C	1	2	3†	4	5	6
1	96 2	95 2	96 0	96 4	96 8	95 2
3	140 4	151 9	140 5	152 3	153 5	152 2
4	106 0	113 3	105 9	113 0	111 3	113 0
5	43 2	30 3	42 9	29 4	30 9	30 4
6	81 4	29 6	81 3	39 6	39 3	29 6
7	129 3	40 4	134 8	78 9	73 1	40 3
8	147 6	80 4	142 5	43 6	151 2	80 4
9	47 1	51 4	47 7	41 7	44 1	51 4
10	60 3	23 7	63 8	13 9	113 9	23 8
11		170 6		170 4	170 3	171 6
OMe		52 6		52 6	52 7	
1′	99 2	99 1	99 3	99 1	99 3	99 1
2′	73 5	73 4	73 6	73 4	73 5	73 4
3′	76 5	76 5	76 5	76 5	76 5	76 4
4′	70 4	70 4	70 4	70 2	70 4	70 4
5′	76 9	77 1	77 0	77 1	77 2	77 0
6′	61 5	61 5	61 5	61 5	61 5	61 5

*Spectra were recorded at 22 6 MHz in D_2O and have been corrected (δC-6′ = 61 5 ppm) [15]

†Additional absorptions at δ169 2 (1C), 132 7 (1C), 130 3 (2C), and 129 6 (3C)

M arvense Whole frozen plants (500 g) were worked up in EtOH as earlier described [15, 16] to give an iridoid-containing fraction (5 6 g) Fractionation on silica gel with $CHCl_3$–MeOH (4 1 and 3 1) gave melampyroside (**3**, 440 mg, 0 1%), an intermediate fraction (1 6 g), and aucubin (**1**, 3 0 g, 0 6%) Reversed phase HPLC of the middle fraction showed that it was a mixture of three compounds Separation of these was effected using a Merck Lobar RP-8 column (H_2O–MeOH, 3 1, UV-detection) The column was loaded with 0 4 g portions and gardoside methyl ester (**5**, 220 mg, 0 05%) was obtained The two remaining compounds were only partly separated to give pure 8-epiloganin (**4**, 90 mg), pure mussaenoside (**2**, 390 mg), and an unresolved mixture (0 9 g) Compounds **1**–**3** were characterized by their NMR spectra (Table 1)

Gardoside methyl ester (**5**) The sample above was passed through activated C in MeOH and the solvent evaporated to give a foam $[\alpha]_D^{20}$ −46° (MeOH, *c* 0 3), ^{1}H NMR (270 MHz, D_2O) δ7 48 (*s*, H-3), 5 48 (*d*, *J* = 3 7 Hz, H-1), 5 39 (*d* (*br*), *J* = 1 7 Hz, CH_2-10), 4 78 (*d*, *J* = 7 9 Hz, H-1′), 4 48 (*t* (*br*), *J* = 6 6 Hz, H-7), 3 74 (*s*, OMe), 3 12 (*m*, H-5 and H-9), 2 06 (*m*, $J_{6\beta\ 6\alpha}$ = 12 9 Hz, $J_{6\beta\ 7}$ = 6 7 Hz, $J_{6\beta\ 5}$ = 2 7 Hz, H-6β) 1 96 (*m*, $J_{6\beta\ 6\alpha}$ = 12 9 Hz, $J_{6\alpha\ 7}$ = 6 9 Hz, $J_{6\alpha\ 5}$ = 3 8 Hz, H-6α), similar, except for the OMe-absorption, to that reported for gardoside [9] [Found C, 50 48, H, 6 45 $C_{17}H_{24}O_{10}$ H_2O requires C, 50 24, H, 6 45%] Acetylation of **5** (Ac_2O–pyridine, 2 hr at 20°) gave the pentaacetate, mp (EtOH) 111–112°, $[\alpha]_D^{20}$ −69° ($CHCl_3$, *c* 0 5) (lit [9] mp 110–111 5°, $[\alpha]_D^{22}$ −75° ($CHCl_3$, *c* 0 7)

8-*Epiloganin* (**4**) The above sample was treated with activated C in MeOH to give the pure compound as a foam $[\alpha]_D^{20}$ −123° (MeOH, *c* 0 5) (lit [13] $[\alpha]_D^{25}$ −101° (MeOH, *c* 1 7)), ^{1}H NMR (270 MHz, D_2O) δ7 48 (*s*, H-3) 5 58 (*d*, *J* = 3 0 Hz, H-1), 4 78 (*d*, *J* = 7 9 Hz, H-1′), 3 88 (*q*, *J* = 5 4 Hz, H-7), 3 75 (*s*, OMe), 3 05 (*dt*, *J* = 8 7, 8 7 and 5 7 Hz, H-5), 2 71 (*dt*, *J* = 8 9, 8 9 and 3 0 Hz, H-9), 2 19 (*m*, *J* = 5 5, 8 8, 5 4 and 7 2 Hz, H-8), 2 04 (*m*, *J* = 5 2, 8 4 and 13 8 Hz, H-6β), 1 90 (*dt*, *J* = 5 7, 5 7 and 13 9 Hz, H-6α), 1 03 (*d*, *J* = 7 3 Hz, H-10), in good agreement with that reported for 8-epiloganin [8] [Found C, 49 02, H, 6 75 Calc for

$C_{17}H_{26}O_{10}$, $1\frac{1}{2}$ H_2O C, 48 91, H, 7 00%]

Catalytic hydrogenation of **5** Compound **5** (115 mg) in EtOH (5 ml) was hydrogenated for 2 hr over Rh–C (38 mg, 5%) After filtration the product (116 mg) was separated (RP chromatography) into **5** (3 mg) and a *ca* 10 1 mixture (56 mg) of 8-epiloganin (**4**) and loganin The ^{1}H NMR spectrum of the major component was identical to that of **4**, but signals arising from loganin were recognized at δ5 50 (*d*, J = 3 5 Hz, H-1) and 1 14 (*d*, J = 7 Hz, H-10)

M cristatum Frozen plant material (250 g) was worked up in EtOH to give *ca* 5 g of crude extract This was separated on a 2 kg, home-made, reversed phase (C-18) silica gel column (H_2O–MeOH, 10 1 → 1 1), yielding salts of iridoid acids (940 mg), aucubin (**1**, 370 mg, 0 15%), gardoside methyl ester (**5**, 74 mg, 0 03%), 8-epiloganin (**4**, 95 mg, 0 04%) and melampyroside (**3**, 58 mg, 0 02%) The fraction containing **6** was acidified with IR-120 and applied to the same column Elution with H_2O–MeOH (3 1) gave mussaenosidic acid (**6**, 700 mg, 0 3%) Treatment with activated C gave **6** as a foam $[\alpha]_D^{20}$ −118° (MeOH, *c* 0 7), ^{1}H NMR (90 MHz, D_2O) δ7 44 (*s*, H-3), 5 55 (*d*, J = 3 Hz, H-1), 2 32 (*dd*, J = 3 Hz and 10 Hz, H-9), and 1 32 (*s*, Me-10), superimposable on the spectrum of mussaenoside (**2**), except for the methyl ester signal of the latter [Found C, 48 84, H, 6 57 $C_{16}H_{24}O_{10}$, H_2O requires C, 48 73, H, 6 65%]

Saponification of mussaenoside Compound **2** (1 05 g) was dissolved in 1N NaOH and kept overnight Acidification with IR-120 gave **6** (0 96 g) indistinguishable from the product above (^{1}H NMR and HPLC)

Acetylation of mussaenosidic acid under forcing conditions (Ac_2O–pyridine, 20 hr at 70°) gave the crystalline pentaacetate, mp (EtOH) 174°, $[\alpha]_D$ −78° ($CHCl_3$, *c* 1 1), ^{1}H NMR (90 MHz, $CDCl_3$) δ7 45 (*s* (*br*), H-3), 5 71 (*d*, J = 2 Hz, H-1), 2 99 (*m*, H-5), 2 67 (*dd*, J = 2 Hz and 9 Hz, H-9) *ca* 2 (5 × OAc, CH_2-6 and CH_2-7), and 1 49 (*s*, Me-10) [Found C, 51 92, H, 5 80 $C_{26}H_{34}O_{15}$, H_2O requires C, 51 65, H, 6 00%]

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