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IRIDOIDS FROM *GALIUM MOLLUGO**

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Key Word Index—*Galium mollugo*, Rubiaceae, iridoid glucoside, secoiridoid glucoside, 10-hydroxyloganin, gardenosidic acid, 10-hydroxymorroniside, NOE

Abstract—From *Galium mollugo*, two new iridoids, gardenosidic acid and 10-hydroxymorroniside as well as 10-hydroxyloganin have been isolated, along with seven known iridoids, secogalioside, asperuloside, asperulosidic acid, daphylloside, monotropein, scandoside and scandoside methyl ester 10-Hydroxyloganin, a compound which was previously considered to be the key biosynthetic intermediate of secoiridoids, was obtained for the first time from a natural source

INTRODUCTION

It has been reported that *Galium mollugo* L. (*G album* Mill) contains the following iridoid glucosides asperuloside (**1**) [1, 2], asperulosidic acid (**2**), monotropein (**3**), galioside (monotropein methyl ester) (**4**) [2] and mollugoside (**5**) [3], as well as a secoiridoid glucoside secogalioside (**6**) [1] No other instance has so far been recorded of the co-occurrence of a secoiridoid glucoside with iridoid glucosides possessing a highly oxidized cyclopentan ring The present paper deals with a re-examination of the iridoid constituents of *G mollugo* plants §

RESULTS AND DISCUSSION

The methanolic extract of the fresh aerial parts of *G mollugo* was fractionated by a combination of charcoal CC, silica gel CC and prep TLC As a result, two new iridoids, gardenosidic acid (**7**) and 10-hydroxymorroniside (**8**), as well as 10-hydroxyloganin (**9**) were isolated The latter glucoside (**9**), which had already been chemically prepared [4–6] was obtained for the first time from a natural source Additionally, three known iridoids scandoside (**10**), scandoside methyl ester (**11**) and daphylloside (**12**) were newly isolated from this plant, besides the reported iridoids asperuloside (**1**), asperulosidic acid (**2**), monotropein (**3**) and secogalioside (**6**)

Gardenosidic acid (**7**)‖

This substance was purified as its methyl ester acetate, a white powder, $C_{29}H_{36}O_{17}$, $[\alpha]_D^{18}$ −70 7° (MeOH, *c* 1 15), which was identical in all respects with an authentic specimen of gardenoside hexaacetate (**13**) [7, 8]

10-Hydroxyloganin (**9**)‖ *and 10-hydroxymorroniside* (**8**)‖

A mixture of these two glucosides was separated after acetylation to give 10-hydroxyloganin hexaacetate (**14**), (7*R*)-10-hydroxymorroniside hexaacetate (**15**) and (7*S*)-10-hydroxymorroniside hexaacetate (**16**)

10-Hydroxyloganin hexaacetate (**14**), a white powder, $C_{29}H_{38}O_{17}$ H_2O, $[\alpha]_D^{22}$ −52 4° ($CHCl_3$, *c* 0 42), showed spectral data (see below) in accord with those of 10-hydroxyloganin hexaacetate which was chemically prepared [4–6] UV λ_{max}^{EtOH} nm (log ε) 232 (4 02), IR ν_{max}^{KBr} cm^{-1} 1755, 1740, 1705 (sh), 1640, ^1H NMR ($CDCl_3$) δ 1 92–2 09 (6 × OAc), 3 06 (*ddd*, *J* = 14 0, 8 0, 1 5 Hz, H-5), 3 70 (*s*, COOMe), 4 06–4 36 (*m*, 10-H_2, 6′-H_2), 5 26 (*d*, *J* = 3 0 Hz, H-1), 7 33 (*d*, *J* = 1 2 Hz, H-3) The Zemplén reaction of the acetate **14** gave a powdery free glucoside, $[\alpha]_D^{22}$ −58 9° (MeOH, *c* 0 89), which was identical in all respects with synthetic 10-hydroxyloganin (**9**) [4–6] Compound **9** was originally considered to be the key biosynthetic intermediate between loganin and secologanin [9], but this possibility was recently ruled out [10, 11]

(7*R*)-10-Hydroxymorroniside hexaacetate (**15**) and (7*S*)-10-hydroxymorroniside hexaacetate (**16**), each $C_{29}H_{38}O_{18}$, were obtained in a 5 1 ratio The physical properties of **15** and **16** (shown below) suggested that **15** and **16** were closely related to (7*R*)-morroniside pentaacetate (**17**) and (7*S*)-morroniside pentaacetate (**18**), respectively (7*R*)-Isomer (**15**), a white powder, $[\alpha]_D^{12}$ −68 1° ($CHCl_3$, *c* 1 09), UV λ_{max}^{EtOH} nm (log ε) 235 (4 09), IR ν_{max}^{KBr} cm^{-1} 1730, 1700, 1630, ^1H NMR ($CDCl_3$) δ 2.01–2 10 (6 × OAc), 2 90 (*dt*, *J* = 13 0, 5 0 Hz, H-5), 3 73 (*s*, COOMe), 4 00 (*m*, H-8), 4 08–4 40 (*m*, 10-H_2, 6′-H_2), 5 66 (*d*, *J* = 9 0 Hz, H-1), 5 74 (*dd*, *J* = 10 0, 2 5 Hz, H-7), 7 44 (*s*, H-3) (7*S*)-Isomer (**16**), colourless needles, mp 183 5°, $[\alpha]_D^{27}$ −101 3° ($CHCl_3$, *c* 1 00), UV λ_{max}^{EtOH} nm

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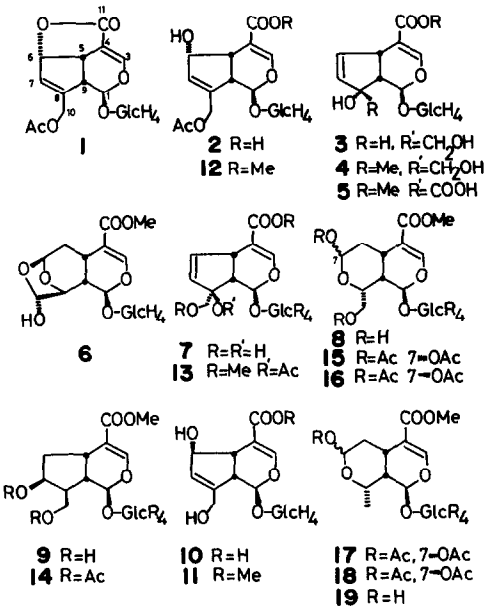
§In the present work, we were unable to detect galioside (**4**) and mollugoside (**5**) This might be due to aerial or seasonal variations in the metabolism of the plant

‖As the ^1H NMR spectrum of the fraction containing **7** did not show any signals from methoxy or acetyl groups, and that of the mixture of **8** and **9** showed no signals from acetyl groups, **7** was purified as its methyl ester acetate, and **8** and **9** were separated as their acetates



(log ε) 235 (4 02), IR ν_{max}^{KBr} cm^{-1} 1710, 1680, 1615, ^{1}H NMR ($CDCl_3$) δ2 00–2 12 (6 × OAc), 3 12 (*dt*, *J* = 12 5, 5 0 Hz, H-5), 3 73 (*s*, COOMe), 4 10–4 42 (*m*, H-8, 10-H_2, 6'-H_2), 5 66 (*d*, *J* = 9 0 Hz, 1-H), 6 16 (*d* (*br*), *J* = 3 0 Hz, H-7), 7 44 (*s*, H-3) In keeping with the above suggestion, the ^{13}C NMR spectra (Table 1) of **15** and **16** were in good accord with those of **17** and **18**, respectively, except that the 10-methyl signals (**17** δ18 8, **18** δ18 8) were replaced by 10-hydroxymethyl signals (**15** δ64 6, 16 δ64 8) Accordingly, it was presumed that **15** was the 10-acetoxymethyl congener of (7*R*)-morroniside penta-acetate (**17**), whereas **16**, that of (7*S*)-morroniside penta-acetate (**18**) This presumption received support from the following ^{1}H and ^{13}C NMR analyses in the ^{1}H NMR spectrum of **15**, strong NOEs were observed between H-5, H-7 and H-8, indicating that these three protons in the chair tetrahydropyran ring of **15** were in a 1,3,5-triaxial relationship Thus, the chirality at both C-7 and C-8 of 15 was proved to be *R* Furthermore, in the ^{13}C NMR spectra, the C-5 and C-8 of **16** resonated 4 1 and 6 4 ppm upfield relative to the corresponding carbons of **15** Such discrepancy of the carbon chemical shifts was also observed between the C-5 and C-8 signals of **18** and **17** The observed upfield shift of C-5 and C-8 was explained in terms of reciprocal γ effects of the C-7 acetoxy group on these carbons in the tetrahydropyran ring of **16** and **18** *

Zemplén reaction of the major product **15** yielded 10-hydroxymorroniside (**8**), $C_{17}H_{26}O_{12}$ $2H_2O$, $[\alpha]_D^{22}$ −97 8° (MeOH, *c* 0 54) as a white powder The accompanying spectral data of **8** clearly indicated that **8** exists, at least in methanol solution, as a mixture of (7*R*)- and (7*S*)-isomers in a 2 1 ratio in the same way as morroniside (**19**) [12] UV λ_{max}^{MeOH} nm (log ε) 238 (4 19), IR ν_{max}^{KBr} cm^{-1} 3350, 1700 (sh), 1690, 1646 (sh), 1625, ^{1}H NMR (CD_3OD) (7*R*)-isomer, δ1 23 (*m*, H-6_{ax}), 1 93 (*m*, H-9), 2 10 (*ddd*, *J* = 13 5, 4 0, 2 0 Hz, H-6_{eq}), 2 85 (*dt*, *J* = 12 5, 5 0 Hz, H-5), 3 70 (*s*, COOMe), 5 78 (*d*, *J* = 9 5 Hz, H-1), 7 50 (*s*, H-3) (7*S*)-Isomer, δ1 54 (*dt*, *J* = 13 5, 3 5 Hz, H-6_{ax}), 1 93 (*m*, H-9, H-6_{eq}), 3 70 (*s*, COOMe), 4 45 (*m*, H-8), 5 28 (*d*, *J* = 3 5 Hz, H-7), 5 84 (*d*, *J* = 9 5 Hz, H-1), 7 50 (*s*, H-3)

*This finding suggests that the tetrahydropyran ring of both compounds also assumes a chair conformation, although we previously surmised that the tetrahydropyran ring of **16** is in a boat form [12]

Table 1 ^{13}C NMR data* of compounds **15**–**18** (50 10 MHz, $CDCl_3$, TMS as int standard)

C	15	16	17	18
1	94 5*d*	94 0*d*	94 8*d*	94 4*d*
3	152 4*d*	152 4*d*	152 5*d*	152 5*d*
4	109 7*s*	110 4*s*	110 3*s*	111 1*s*
5	30 4*d*	26 3*d*	30 1*d*	26 0*d*
6	33 5*t*	31 8*t*	33 1*t*	31 3*t*
7	94 0*d*	90 9*d*	93 9*d*	91 3*d*
8	75 8*d*	69 4*d*	73 6*d*	67 3*d*
9	36 5*d*	36 6*d*	38 9*d*	39 2*d*
10	64 6*t*	64 8*t*	18 8*q*	18 8*q*
11	166 3*s*	166 4*s*	166 4*s*	166 6*s*
OMe	51 5*q*	51 4*q*	51 3*q*	51 3*q*
1'	97 4*d*	97 2*d*	96 8*d*	96 8*d*
2'	71 0*d*	71 0*d*	71 0*d*	71 1*d*
3'	72 2*d*	72 2*d*	72 1*d*	72 1*d*
4'	68 8*d*	68 4*d*	68 6*d*	68 7*d*
5'	72 6*d*	72 5*d*	72 6*d*	72 6*d*
6'	61 8*t*	61 5*t*	61 8*t*	61 7*t*

*Off resonance patterns are given after the chemical shift value Each compound has additional signals arising from acetoxy groups

EXPERIMENTAL

General procedure Mps uncorr, ^{1}H NMR (200 MHz) and ^{13}C NMR (50 10 MHz) $CDCl_3$ or CD_3OD with TMS as int standard, TLC silica gel 60 GF_{254}, spots visualized by irradiation with UV light (254 nm), exposure to I_2 vapour or spraying with anisaldehyde–H_2SO_4 reagent (anisaldehyde 0 5 ml, 95% EtOH 9 0 ml and AcOH 0 1 ml) followed by heating, Prep TLC silica gel 60 PF_{254}, bands detected by irradiation with UV light (254 nm) The bands due to free glucosides were extracted with $CHCl_3$–MeOH (7 3), while the ones due to the acetates were extracted with $CHCl_3$–MeOH (19 1), CC carbon (Wako and Takeda) or silica gel PF_{254}

Plant material The seeds of *Galium mollugo* were donated by Drs S R Jensen and B J Nielsen (Technical University of Denmark) They were planted at the Medicinal Plant Garden, Faculty of Pharmaceutical Sciences, Kyoto University in October 1980, and the plants were collected in July 1981 A voucher specimen of *G mollugo* (H Inouye No 1) has been deposited in the Herbarium of the Institute of Botany, Faculty of

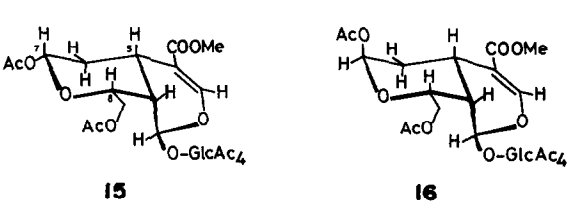


Fig 1 Conformation of compounds **15** and **16**

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Isolation of iridoids Fresh aerial parts (11 9 kg) were extracted with boiling MeOH (50 l × 3) After concentration of the combined extracts *in vacuo* at 40°, the residue was diluted with H_2O (15 l), and the insoluble material was filtered off through a Celite layer, which was then washed with H_2O (10 l) The combined filtrate and washings were concentrated *in vacuo* to give a residue (344 5 g) which was chromatographed on a charcoal (1 4 kg) column developed with EtOH–H_2O mixtures containing increasing amounts of EtOH This gave the following fractions Fr 1, the faster 10% EtOH (72 5 g) Fr 2, slower 10% EtOH (7 9 g) Fr 3, 40% EtOH (8 0 g) Fr 4, 80% EtOH (3 0 g) Fr 1 was extracted with boiling EtOH (100 ml × 3) and the residue (1 7 g) of the EtOH extract treated with excess ethereal CH_2N_2 in MeOH at 0° for 15 min The resultant ppt was filtered off and washed with MeOH (10 ml × 3) The combined filtrate and washings were concentrated *in vacuo* to give a residue (1 05 g), which was subjected to silica gel (30 g) CC with $CHCl_3$–MeOH of an increasing MeOH content The residue (184 mg) of the 10% MeOH–$CHCl_3$ eluate was subjected to prep TLC with $CHCl_3$–MeOH (4 1, developed × 3) The less mobile major band afforded crude glucoside (46 mg) This was acetylated with pyridine–Ac_2O (each 0 5 ml) at 30° for 15 hr and the product (60 mg) purified by prep TLC (four developments) with C_6H_6–Et_2O (3 1) to yield gardenoside hexaacetate (**13**) (16 mg) as a white powder An aliquot (2 57 g) of Fr 2 was rechromatographed on a charcoal (15 g) column developed successively with H_2O (1 l), 7 5% MeOH (1 l) and MeOH (1 l) The MeOH eluate afforded a mixture (180 mg) of monotropein (**3**) and scandoside (**10**), which was successively methylated and acetylated in the conventional way The product was then subjected to prep TLC ($CHCl_3$–MeOH, 20 1) Of two major bands, the more mobile one gave the hexaacetate (130 mg) of scandoside methyl ester (**11**) as colourless needles, mp 133–135°, whereas the less mobile band furnished the pentaacetate (18 mg) of monotropein methyl ester (**4**) as colourless needles, mp 148–153° An aliquot (5 g) of Fr 3 was chromatographed on a silica gel (120 g) column with $CHCl_3$–MeOH of an increasing MeOH content The 5% MeOH–$CHCl_3$ eluate gave first crystalline asperuloside (**1**, 1600 mg) and secondly powdery secogalioside (**6**, 111 mg) The 7 5% MeOH–$CHCl_3$ eluate furnished a residue (47 mg), which was subjected to prep TLC ($CHCl_3$–MeOH, 4 1, developed × 3) to afford scandoside methyl ester (**11**, 9 mg) as a white powder The 10% MeOH–$CHCl_3$ eluate gave a residue (1530 mg), an aliquot (471 mg) of which was submitted to prep TLC ($CHCl_3$–MeOH, 4 1, developed × 4). Of two major bands, the more mobile one gave a glucoside fraction A (254 mg), whereas the less mobile one, a glucoside fraction B (110 mg) Fraction A was acetylated and the product (336 mg) subjected to prep TLC (C_6H_6–Et_2O, 7 3, developed × 5) to give 10-hydroxyloganin hexaacetate (**14**, 113 mg), (7*R*)-10-hydroxymorroniside hexaacetate (**15**, 89 mg), and (7*S*)-10-hydroxymorroniside hexaacetate (**16**, 22 mg) in order of decreasing polarity **14**, Found C, 51 49, H, 5 74 $C_{29}H_{39}O_{17}$ H_2O requires C, 51 48, H, 5 96% **15**, Found C, 51 79, H, 5 76 $C_{29}H_{38}O_{18}$ requires C, 51 62, H, 5 68% **16**, Found C, 51 13, H, 5 81 $C_{29}H_{38}O_{18}$ requires C, 51 62, H, 5 68% Next, fraction B (110 mg) was subjected to prep TLC ($CHCl_3$–MeOH, 4 1, developed × 4), and the major band gave a white powder (78 mg), which was recrystallized from EtOH to afford asperulosidic acid (**2**, 55 mg) as colourless needles, mp 125–128° Fr 4 was chromatographed on a silica gel (60 g) column with $CHCl_3$–MeOH of an increasing MeOH content The residue (388 mg) of the 5% MeOH–$CHCl_3$ eluate was subjected to prep TLC ($CHCl_3$–MeOH, 4 1, developed × 3) The major band gave daphylloside (**12**, 207 mg) as a white powder

Zemplén reaction of 10-hydroxyloganin hexaacetate (**14**) Methanolic NaOMe (0 1 M, 0 1 ml) was added to a soln of **14** (50 mg) in MeOH (2 ml) and the mixture was refluxed for 10 min After cooling, the soln was neutralized with Amberlite IR-120B (H^+-form) and concd *in vacuo* The residue (45 mg) was purified by prep TLC ($CHCl_3$–MeOH, 4 1) to give 10-hydroxyloganin (**9**) as a white powder ^1H NMR (CD_3OD) δ1 54 (*ddd*, J = 14 0, 10 0, 4 5 Hz, H-6_{ax}), 2 08 (*m*, H-8, H-9), 2 26 (*ddd*, J = 14 0, 7 0, 1 5 Hz, H-6_{eq}), 4 30 (deformed *t*, H-7), 5 16 (*d*, J = 6 0 Hz, H-1), 7 46 (*d*, J = 1 2 Hz, H-3) (Found C, 49 26, H, 6 45 $C_{17}H_{26}O_{11}$ $\frac{1}{2}$ H_2O requires C, 49 15, H, 6 55%)

Zemplén reaction of (7R)-10-*hydroxymorroniside hexaacetate* (**15**) Methanolic NaOMe (0 1 M, 0 1 ml) was added to a soln of **15** (107 mg) in MeOH (1 ml) and the mixture was allowed to stand for 30 min in iced H_2O The soln was worked up in the same way as above to afford 10-hydroxymorroniside (**8**, 52 1 mg) as a white powder (Found C, 44 71, H, 6 58 $C_{17}H_{26}O_{12}$ $2H_2O$ requires C, 44 54, H, 6 60%)

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