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MOLLUGOSIDE, AN IRIDOID GLUCOSIDE FROM *GALIUM MOLLUGO*

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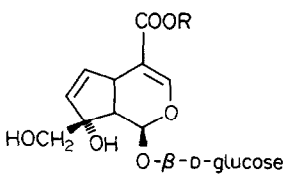
Key Word Index—*Galium mollugo*, Rubiaceae, iridoid glucosides, mollugoside, 8α-hydroxy-apodanthoside, anionization of monotropein and mollugoside, mono- and disodic salts

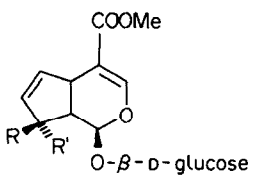
Abstract—Mollugoside, a new iridoid glucoside isolated from *Galium mollugo*, has been characterized as the 8α-hydroxyapodanthoside by simple chemical transformations and comparison of ^{13}C NMR spectra of mollugoside and monotropein with those of corresponding carboxilate anions

INTRODUCTION

A previous investigation on *Galium mollugo* [1] reported the isolation of four iridoids asperuloside, asperulosidic acid, monotropein (**1**) and galioside (**2**) A re-examination of the plant, collected in the spring and extracted with an improved technique, showed the presence of an acidic polar iridoid glucoside This new iridoid, present in the plant in small amount, has been named mollugoside (**3**)



1 R = H
2 R = Me



3 R = COOH, R' = OH
4 R = COOMe, R' = OH
5 R = COOH, R' = H
9 R = OH, R' = CH_2OH

RESULTS AND DISCUSSION

A new technique was developed employing mild conditions for the extraction of the iridoid fraction plant materials Instead of ethanol, a concentrated solution (25%) of sodium chloride was used as the solvent and it has the following advantages (1) it is inexpensive, (2) it gives higher yields and is more rapid than ethanol (the vanillin test for iridoids becomes positive after a few minutes and in a few hours the extraction is complete), (3) chlorophyll and other apolar materials are not extracted, (4) it is very useful for extracting very polar compounds The work-up of the final saline solution is carried out, as usual, by absorption on charcoal and successive elution of salts with water and of iridoid components with mixtures water–ethanol with an increasing content of ethanol

Compound **3** is an amorphous powder with acidic properties, molecular formula $C_{17}H_{21}O_{12}$ The UV absorption (230 nm, log ε 3 5) indicated the presence of a conjugated enol–ether system Enzymatic hydrolysis with β-glucosidase followed by isolation of D-glucose confirmed that **3** was a β-D-glucopyranoside The ^1H NMR spectrum of **3** also showed the presence of a doublet (δ 4 82, J = 7 5 Hz), characteristic for the anomeric proton of a β-D-glucopyranose Furthermore, this spectrum closely resembled that of **2** [1] except for the lack of signals of the hydroxymethyl group at C-8 and the downfield shift (0 34 ppm) of the H-9 resonance, both indicating the probable presence of a COOR group linked to the hydroxylated quaternary C-8

Chemical support for the presence of a free carboxyl group in **3** was provided by the preparation of a bis-methylester (**4**) which showed in the ^1H NMR spectrum a signal at δ 3 84 of a second methoxycarbonyl group

The substitution pattern (OH, COOR) at C-8 of **3** was confirmed by comparison of its ^{13}C NMR spectrum (Table 1) with that of apodanthoside **5** [2] The strong deshielding of C-8 in **3** ($\Delta\delta = \sim 34$) is clearly due to the presence on the same carbon of an additional hydroxyl group which is also responsible for the smaller shift differences observed for C-6, C-7, C-9 and C-10 of these compounds These preliminary data suggested that **3** may be the 8-hydroxy derivative of **5**, with the possibility of a reversed arrangement between the carboxyl and carboxymethyl groups

The problem of the correct location (at C-4 or C-8) of the free carboxyl group of **3** was clarified by comparing the ^{13}C NMR chemical shift values (Table 2) of the carboxyl or carboxymethyl carbons in a series of 28 iridoid glucosides bearing one of these functions at C-4 and, in some cases, also at C-8 The analysis of these data established the chemical shift ranges observed for the carbons of the carboxyl and carboxymethyl functions at C-4 (δ 166 6–172 6, C=O, 49 1–52 9 OMe) or C-8 (175 4–176 8, C=O, 53 9, OMe)

The values observed for the resonances of the carboxyl and methoxyl carbons of **3** were in agreement with the location of the carboxymethyl group at C-4 (signals at δ 170 26 and 52 64) and of the carboxyl group at C-8 (182 10) The deshielding of *ca* 6 ppm observed for the carbonylic C-10 of **3** with respect to **5** is evidently caused by interaction (hydrogen bonding) with the geminal C-8 hydroxyl group These assignments were confirmed by the resonance value (δ 54 2) of the new methoxyl signal which appeared in the spectrum of **4**

Table 1 ^{13}C NMR chemical shift assignments for compounds 1–9*

Carbon No	1	2§	3	4	5†	6	7	8	9§
C-1	95 20	95 12	94 72	94 08	95 0	94 72	94 74	94 33	94 35
C-3	152 44	151 96	151 81	151 96	150 4	146 89	151 77	146 86	151 88
C-4	111 04	111 03	110 96	110 46	108 6	116 53	110 95	116 21	111 13
C-5	37 89	37 81	37 24	37 52	38 0	39 24	37 31	38 69	37 98
C-6	132 82	132 81	133 23	131 26	128 4	132 13	133 39	132 62	134 56
C-7	138 01	137 99	138 28	139 94	132 3	138 80	138 18	138 96	136 04
C-8	85 65	85 55	86 62‡	85 55	52 7	85 85	86 26	86 62	86 13
C-9	44 84	44 82	47 46	47 08	42 3	45 00	47 39	47 68	51 38
C-10	67 39	67 39	182 10‡	176 08	176 8	67 40	181 26	181 73	66 11
				54 21					
C-11	171 30	170 22	170 26	169 94	166 6	175 70	170 13	175 69	170 11
		52 66	52 64	52 73	49 1		52 67		52 74
C-1′	99 13	99 12	99 05	98 94	97 7	98 86	99 05	98 86	99 23
C-2′	73 53	73 45	73 42	73 44	71 7	73 48	73 52	73 53	73 53
C-3′	76 47	76 47	76 46	76 39	74 9	76 40	76 48	76 46	76 48
C-4′	70 42	70 34	70 38	70 33	68 5	70 33	70 43	70 50	70 49
C-5′	77 15	77 06	77 05	77 06	75 3	77 04	77 08	77 05	77 15
C-6′	61 54	61 52	61 51	61 45	59 7	61 51	61 54	61 61	61 63

*The spectra of 1–4 and 6–9 were determined in D_2O and were obtained at 20 MHz and 22 63 MHz in the Fourier transform mode Chemical shifts are expressed according to the following equation $\delta^{TMS} = \delta^{Dioxane} + 67\,4$ ppm

†Taken from ref [2] and determined in CD_3OD

‡Line broadened and of low intensity

§Comparison with corresponding spectra in CD_3OD [6] evidenced only small shift changes due to the solvent effect

To demonstrate unambiguously that C-8 was the linkage position of the free carboxylic function of **3** we performed the anionization of **3** and **1**, (the latter was used as a model compound), by titration with 0 1 N sodium hydroxide solution (monosodic salts of **3** and **1**) and saponification of the carboxymethyl function of **3** (disodic salt) Successive comparisons of the ^{13}C NMR spectra (Table 1) of **1**, **3**, monotropein sodic salt (**6**), mollugoside monosodic salt (**7**) and mollugoside disodic salt (**8**) showed clearly that in the conversion of **3** into **7** only slight and hardly diagnostic shift changes are measurable (Table 3) for carbons close to the site of the negative charge in contrast with the appreciable deshielding values depicted for all centers around the ionization site of saturated carboxylic acids [3]

By contrast comparison of the ^{13}C NMR chemical shifts of the pair **3** and **8** revealed marked effects, induced by anionization, on C-11 (+ 5 43 ppm), C-4 (+ 5 25 ppm) and C-3 (− 4 95 ppm) but smaller effects on C-5 (+ 1 45 ppm) and C-1 (− 0 39 ppm) These $\Delta\delta$ values are in perfect agreement with the corresponding ones observed in the model pair **1** and **6**, owing to the ionization of the C-4 carboxyl group, and in acid-salt pairs relative to simple α, β-olefinic acids [4] This good correspondance provides the definitive proof of the correct location of the carboxyl and carboxymethyl functions in **3** A parallel analysis of the ^1H NMR spectra (see Experimental) of the same pairs showed that the anionization of carboxyl groups causes the shielding of the olefinic H-3 (0 36 ppm in **3** → **8**, 0 34 ppm in **1** → **6**) as the unique diagnostic shift change

Finally, the stereochemistry at the C-8 centre of **3** was demonstrated to be of the 'monotropein-type', on the basis of the 'C-8 epimers rule' [5, 6] (an α-hydroxy group at C-8 exerts on C-9 a shielding of 5–7 ppm with respect to its β counterpart) Comparison of the chemical shift value of C-9 of **3** (Table 1) with that of the corresponding carbon in the pair of C-8 epimers galioside (**2**) and gardenoside (**9**), indicated that the configuration at the C-8 centre of **3** was β-carboxyl, α-hydroxyl This stereochemistry was further supported by the better agreement of C-6 and C-7 resonances of **3** (δ 133 23 and 138 28) with the corresponding ones of **2** (132 81 and 137 99) than with those of its C-8 epimer **9** (134 56 and 136 04) as well as by biosynthetic considerations (co-occurence in the plant of **1** and **2** of which **3** may be considered an oxidation product)

EXPERIMENTAL

General techniques were as described earlier [5]

Isolation of the iridoid fraction The aerial parts (4 3 kg fr wt) of *Galium mollugo* were collected in the meadows of the Botanical Garden of the University of Rome in April 1981 (voucher deposited at the Botanical Institute Herbarium of this University) After chopping, the plant material was extracted twice at room temp with 25% NaCl (8 l) for 2 days each The extracts were combined and decolorizing charcoal was added until the suspension showed a negative vanillin test A small fraction (50 ml) of the suspension was stratified on a Gooch funnel (2 cm diam) and eluted with H_2O to remove salts and then with EtOH PC of the EtOH soln (developed with *n*-BuOH–HOAc–H_2O, 63 10 27 and visualized with vanillin) showed the same components as the corresponding EtOH extract of fresh leaves R_f 0 45 (violet, asperuloside and asperulosidic acid), 0 37 (violet, galioside, **2**), 0 29 (violet, monotropein, **1**) The remaining charcoal suspension was stratified on a Gooch funnel (20 cm diam) containing a layer of Si gel (100 g) Salts, mono- and disaccharides were removed with H_2O (11 l) and 5% EtOH (13 l) Successive fractions showed a positive vanillin test Fraction A, eluted with 20% EtOH (23 l), Fraction B, 30% EtOH (3 l), Fraction C, 50% EtOH (10 l), Fraction D, 80% EtOH (2 l)

Table 2 ^{13}C NMR chemical shift assignments of C-10 and C-11 carbonylic carbons of iridoids

Compound	Ref	C-10		C-11	
		C=O	OMe	C=O	OMe
Hastatoside	[5]*	—	—	168 3	52 7
Ipolamide	[5]*	—	—	169 0	52 5
Lamide	[5]*	—	—	169 0	52 6
Loganin	[5]*	—	—	170 7	52 6
Phlomiol	[5]*	—	—	168 9	52 7
Pulchelloside I	[5]*	—	—	168 6	52 7
Pulchelloside II	[5]*	—	—	167 3	52 6
Shanzside Me-ester	[5]*	—	—	170 4	52 8
Verbenalin	[5]*	—	—	169 6	52 8
Asperulosidic acid	[6]†	—	—	172 6	OH
Desacetyl asperulosidic acid	[6]†	—	—	170 9	OH
Desoxy loganin	[6]†	—	—	169 7	51 6
Geniposidic acid	[6]†	—	—	171 1	OH
Ladroside	[6]†	—	—	169 3	51 8
Plumieride	[6]†	—	—	168 4	52 0
Scandoside	[6]†	—	—	172 1	OH
Thevinidoside	[6]†	—	—	168 1	51 8
Galioside	*	—	—	170 2	52 7
Gardenoside	[7]*	—	—	170 1	52 7
Monotropein	[7]*	—	—	171 3	OH
8-Epiloganin	[8]*	—	—	170 7	52 6
Aralidioside	[9]*	175 4	53 9	167 9	52 8
Griselinoside	[9]*	176 3	53 9	169 2	52 9
Apodanthoside	[2]†	176 8	OH	166 6	49 1
Desacetyl asperulosidic acid methylester	[2]†	—	—	167 1	50 3
Feretoside	[2]†	—	—	170 1	51 9
Penstemonoside	[10]†	—	—	169 5	51 8
Penstemoside	[10]†	—	—	168 1	51 8

*Solvent, D_2O
†Solvent, CD_3OD

Table 3 Anionization effects on ^{13}C chemical shifts

		C-11	C-4	C-3	C-5	C-1
δ_{acid}	1	171 30	111 04	152 44	37 89	95 20
δ_{anion}	6	175 70	116 53	146 89	39 24	94 72
$\delta_{anion}-\delta_{acid}$		+4 40	+5 49	−5 55	+1 35	−0 48
δ_{acid}	3	170 26	110 96	151 81	37 24	94 72
$\delta_{dianion}$	8	175 69	116 21	146 86	38 69	94 33
$\delta_{dianion}-\delta_{acid}$		+5 43	+5 25	−4 95	+1 45	−0 39

+, Deshielding, −, shielding

Isolation of mollugoside (3) Fraction A chromatographed on Si gel developed with *n*-BuOH satd with H_2O, afforded crude 3 (300 mg) still contaminated by other products, mainly monotropein (1) This residue was rechromatographed on acidic Si gel in CH_2Cl_2–MeOH–H_2O (7 3 0 3) satd with CO_2 to afford pure 3 (205 mg) UV λ_{nm}^{max} (log ε) 230 (3 5), ^1H NMR (D_2O) δ 7 46 (1H, *d*, $J_{3\ 5}$ = 1 3 Hz, H-3), 6 35 (1H, *dd* $J_{5\ 6}$ = 2 7 Hz, $J_{6\ 7}$ = 6 0 Hz, H-6), 5 74 (1H, *dd*, $J_{5\ 7}$ = 1 7 Hz, $J_{6\ 7}$ = 6 0 Hz, H-7), 5 68 (1H, *d*, $J_{1\ 9}$ = 1 3 Hz, H-1), 3 9–3 5 (1H, *m*, H-5), 3 78 (3H, *s*, COOMe-4), 3 12 (1H, *dd*, $J_{1\ 9}$ = 1 3 Hz, $J_{5\ 9}$ = 9 0 Hz, H-9), 4 82 (1H, *d*, $J_{1',2'}$ = 7 5 Hz, H-1')

All chromatographic fractions were checked on Si gel TLC with an acidic eluent (CH_2Cl_2–MeOH–H_2O–HOAc, 7 3 0 2 0 2) and spots located by spraying with 2N H_2SO_4 Compound 3 appeared as a red spot with low R_f value However, 3 showed a very feeble brown reaction with vanillin reagent and for this reason it is practically undetectable on PC (R_f 0 48 in *n*-BuOH–MeOH–HOAc, 63 10 27)

Mollugoside methylester (4) Compound 3 (110 mg) in MeOH was treated with excess CH_2N_2–Et_2O at 0° for 20 min When all 3 reacted (checked by TLC), excess reagent was evaporated and the residue, chromatographed on Si gel in CH_2Cl_2–MeOH–H_2O (7 3 0 3), gave pure 4 (67 mg) $[\alpha]_D^{25}$ = −88 7° (MeOH, *c* 0 83), (Found C 49 86, H 5 72 $C_{18}H_{24}O_{12}$ requires C 50 00, H 5 65 %) ^1H NMR (D_2O) δ 7 48 (1H, *d*, $J_{3\ 5}$ = 1 3 Hz, H-3), 6 45 (1H, *dd*, $J_{5\ 6}$ = 3 0 Hz, $J_{6\ 7}$ = 6 0 Hz, H-6), 5 86 (1H, *dd*, $J_{5\ 7}$ = 1 7 Hz, $J_{6\ 7}$ = 6 0 Hz, H-7), 5 70 (1H, *d*, $J_{1\ 9}$ = 1 7 Hz, H-1), 3 9–3 5 (1H, *m*, H-5), 3 84 (3H, *s*, COOMe-8), 3 78 (3H, *s*, COOMe-4), 3 16 (1H, *br d*, $J_{5\ 9}$ = 9 0 Hz, H-9)

Monotropein (1) ^1H NMR (D_2O) [7] δ 7 40 (1H, *br s*, $J_{3\ 5}$ = 1 0 Hz, H-3), 6 21 (1H, *dd*, $J_{5\ 6}$ = 2 8 Hz, $J_{6\ 7}$ = 5 7 Hz, H-6), 5 68 (1H, *dd*, $J_{5\ 7}$ = 1 7 Hz, $J_{6,7}$ = 5 7 Hz, H-7), 5 60 (1H, *d*, $J_{1\ 9}$ = 2 0 Hz, H-1), 3 63 (2 H, *br s*, 2H-10), 3 6–3 3 (1H, *m*, H-5), 2 66 (1H, *dd*, $J_{1\ 9}$ = 2 0 Hz, $J_{5\ 9}$ = 8 0 Hz, H-9)

Galioside (2) ^1H NMR (D_2O) [1] δ 7 50 (1H, *d*, $J_{3\ 5}$ = 1 3 Hz, H-3), 6 32 (1H, *dd*, $J_{5\ 6}$ = 3 0 Hz, $J_{6\ 7}$ = 5 7 Hz, H-6), 5 78 (1H,

dd, $J_{5,7}$ = 1 7 Hz, $J_{6,7}$ = 5 7 Hz, H-7), 5 72 (1H, *d*, $J_{1,9}$ = 1 9 Hz, H-1), 3 82 (3H, *s*, COOMe-4), 3 64 (2H, *br s*, 2H-10), 3 5–3 0 (1H, *m*, H-5), 2 78 (1H, *dd*, $J_{1,9}$ = 1 9 Hz, $J_{5,9}$ = 8 3 Hz, H-9)

Monotropein sodic salt (**6**) ^{1}H NMR (D_2O) δ 7 06 (1H, *d*, $J_{3,5}$ *ca* 1 0 Hz, H-3), 6 22 (1H, *dd*, $J_{5,6}$ = 2 7 Hz, $J_{6,7}$ = 5 7 Hz, H-6), 5 68 (1H, *dd*, $J_{5,7}$ = 1 7 Hz, $J_{6,7}$ = 5 7 Hz, H-7), 5 56 (1H, *d*, $J_{1,9}$ = 1 9 Hz, H-1), 3 66 (2H, *br s*, 2H-10), 3 9–3 6 (1H, *m*, H-5), 2 66 (1H, *dd*, $J_{1,9}$ = 1 9 Hz, $J_{5,9}$ = 8 0 Hz, H-9)

Mollugoside monosodic salt (**7**) ^{1}H NMR (D_2O) δ 7 46 (1H, *d*, H-3), 6 34 (1H, *dd*, H-6), 5 72 (1H, *dd*, H-7), 5 68 (1H, *d*, H-1), 3 9–3 6 (1H, *m*, H-5), 3 80 (3H, *s*, COOMe-4), 3 10 (1H, *br d* H-9)

Mollugoside disodic salt (**8**) ^{1}H NMR (D_2O) δ 7 10 (1H, *d*, H-3), 6 34 (1H, *dd*, H-6), 5 72 (1H, *dd*, H-7), 5 60 (1H, *d*, H-1), 3 93 6 (1H, *m*, H-5), 3 10 (1H, *br d*, H-9)

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