Phytochemistry, Vol 23, No 10, pp 2313–2315, 1984
Printed in Great Britain

0031–9422/84 $3 00 + 0 00
© 1984 Pergamon Press Ltd

ISOMARTYNOSIDE, A PHENYLPROPANOID GLYCOSIDE FROM *GALEOPSIS PUBESCENS*

IHSAN CALIS*, MOHAMED F LAHLOUB, ERICH ROGENMOSER and OTTO STICHER

Pharmazeutisches Institut, Eidgenossische Technische Hochschule Zurich, ETH-Zentrum, 8092 Zurich, Switzerland

(*Revised received* 28 *March* 1984)

Key Word Index—*Galeopsis pubescens*, Labiatae, phenylpropanoid glycosides, martynoside, isomartynoside, deacyl martynoside, deacyl isomartynoside, ferulic acid, EIMS, ^{1}H and ^{13}C NMR

Abstract—The structure of isomartynoside, a new phenylpropanoid glycoside, isolated along with the known martynoside from *Galeopsis pubescens*, has been elucidated on the basis of chemical and spectral data as 3-hydroxy-4-methoxy-β-phenylethoxy-*O*-α-L-rhamnopyranosyl-(1 → 3)-6-*O*-feruloyl-β-D-glucopyranoside

INTRODUCTION

During a systematic study of iridoidal constituents of *Galeopsis* species, we encountered a noniridoidal fraction in *Galeopsis pubescens* [1] that has now been established to contain two phenylpropanoid glycosides In this paper we describe the isolation and structure determination of these two compounds, martynoside (**1**) and isomartynoside (**2**)

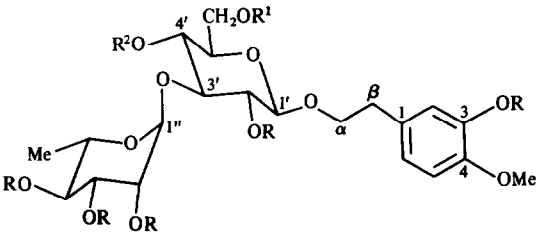
RESULTS AND DISCUSSION

Low pressure liquid chromatography of the appropriate fraction on a reversed phase C-18 column afforded two pure glycosides The faster running compound was identified by spectral means to be a known phenylpropanoid glycoside, martynoside (**1**), previously isolated from *Martynia louisiana* [2] and the slower running one a new compound, named isomartynoside (**2**) The structure determination of the new compound **2** now follows

The molecular formula $C_{31}H_{40}O_{15}$ of **2** is based on electron impact mass spectrometry, the number of signals in the PND ^{13}C NMR and their multiplicities in the SFORD and integration of the ^{1}H NMR spectra of **2** and its peracetate **3** The ^{1}H NMR (300 MHz) spectrum of **2** showed six aromatic protons, the coupling constants of which gave the substitution pattern indicated in the formula, two olefinic protons (*d*, H-α′ and H-β′) which appeared as an AB-system (the coupling constant (J = 16 Hz) indicated a *trans*-geometry), two methoxyl groups, a benzylic methylene and two anomeric protons The coupling constants of the anomeric protons at δ4 33 (J = 7 8 Hz) and 5 17 (J = 1 0 Hz) are consistent with the configurations β for D-glucose and α for L-rhamnose, respectively The region of the remaining glycosidic protons had the expected splitting and integral The ^{1}H NMR spectrum of the heptaacetate **3** showed the presence of five aliphatic and two aromatic acetyl groups and the remaining signals were similarly related

The ^{13}C NMR spectrum of isomartynoside (**2**) showed 30 signals (two methoxyl signals came together) and their assignments are given in Table 1 The assignment of the signals are based on (i) multiplicity of the signal in SFORD spectrum, (ii) literature data on chemical shifts of functional groups and (iii) comparison of the spectral data of **1**, **2** and **3** and related compounds [3, 4] Of 30 (from 31 carbons) signals in **2**, 26 signals are virtually identical to signals observed in the ^{13}C NMR of **1** The ^{13}C NMR signals assigned due to rhamnosyl, feruloyl and 3-hydroxy-4-methoxy-β-phenylethanol moieties were virtually superimposable with that of **1** From these and above data we interprete that isomartynoside (**2**) and martynoside (**1**) differ from each other only in the site of acylation on the glucosyl moiety

In order to locate the site of acylation, the compounds **1** and **2** were separately treated with aq potassium hydroxide to give ferulic acid (identified by chromatography and ^{1}H NMR spectroscopy) and deacyl glycoside **4** The EIMS of **4** revealed the molecular ion peak $[M]^+$ at m/z 476 and further peaks at m/z 330 $[M - \text{rhamnosyl}]^+$, m/z 146 (terminal rhamnose moiety) and m/z 168 (agylcone = 3-hydroxy-4-methoxy-β-phenylethanol) The ^{13}C NMR of **4** showed 21 signals as expected, 19 of which are virtually identical in chemical shift and multiplicity



1 Martynoside R = R^1 = H , R^2 = feruloyl
2 Isomartynoside R = R^2 = H , R^1 = feruloyl
3 Isomartynoside heptaacetate R = R^2 = Ac , R^1 = acetylferuloyl
4 Deacyl martynoside = deacyl isomartynoside R = R^1 = R^2 = H

*Research Associate (January 1982–December 1983) at the Pharm Inst ETH Zurich Present address Faculty of Pharmacy, Department of Pharmacognosy, Hacettepe University, Ankara, Turkey

Table 1 ^{13}C NMR data of martynoside (1), isomartynoside (2) and deacyl-martynoside (4)*

C-Atom	1	2	4
1	132 93	132 84	132 79
2	112 95	112 84	112 77
3	147 57	147 60	147 37
4	147 37	146 92	147 18
5	117 19	117 07	116 96
6	121 31	121 22	121 06
α	72 20	72 22	71 87
β	36 63	36 70	36 46
OMe	56 63	56 23	56 45
1′	104 24	104 41	104 08
2′	76 24	75 72	75 49
3′	81 68	84 14	84 38
4′	70 74	70 60	70 09
5′	76 04	75 42	77 70
6′	62 49	64 78	62 58
1″	103 07	102 79	102 62
2″	72 42	72 40	72 23
3″	72 20	72 40	72 14
4″	73 87	74 07	73 87
5″	70 31	70 13	69 98
6″	18 63	18 03	17 87
1‴	127 71	127 72	
2‴	111 91	111 73	
3‴	149 41	149 52	
4‴	150 81	150 80	
5‴	116 66	116 55	
6‴	124 46	124 35	
α′	115 19	115 33	
β′	148 03	147 17	
CO	168 39	169 00	
OMe	56 63	56 23	

*Run at 75 47 MHz in CD_3OD with TMS as internal standard

(SFORD) with the signals observed in the ^{13}C NMR spectrum of **2** An expected downfield shift ($\Delta\delta = +2\,20$) of the signal of the C-6′ in **2**, due to the acylation, is observed in respect to **4** along with an upfield shift ($\Delta\delta = -2\,28$) of the C-5′ due to the β-effect from the adjacent acyl group at C-6′ Comparison of **2** with **1** showed a downfield shift ($\Delta\delta = +2\,46$) of C-3′ and also shifts of C-5′ and C-6′ (Table 1) confirming thereby the two compounds to be different These differences in the chemical shift values are expected because **2** is acylated at C-6′ and **1** at C-4′ Additional evidence in favour of the acylation at C-6′ is also obtained from the ^{1}H NMR data of **2** The chemical shift values of H_2C-6′ in **2** are also in good agreement with the corresponding signals of similar acylation pattern [15]

Based on these data, the structure of isomartynoside is established as 3-hydroxy-4-methoxy-β-phenyl-ethoxy-*O*-α-L-rhamnopyranosyl-(1 → 3)-6-*O*-feruloyl-β-D-glucopyranoside (**2**)

EXPERIMENTAL

General procedures ^{1}H and ^{13}C NMR spectra [δ(ppm), *J*(Hz)] were obtained at 300 13 MHz (^{1}H NMR) and at 75 47 MHz (^{13}C NMR) using a Bruker WM 300 Spectrospin instrument in Fourier transform mode with tetramethylsilane as an internal standard EIMS (*m/z*) were recorded at 70 eV Silica gel 60 (70–230 mesh, Merck) was used for column chromatography and silica gel 60 F_{254} (Merck) prepared plates for TLC Oph-Up C_{12} (Art No 1022, Antec AG) prepared plates were used for reversed phase TLC The reversed phase material of PrepPAK-500/C-18 cartridges (Waters) was used for the preparative low pressure liquid chromatography

Plant material The plant material was collected near Giornico, Switzerland [1] A voucher specimen is deposited in the Herbarium of the Laboratory of Pharmacognosy and Phytochemistry, School of Pharmacy, ETH Zurich

Extraction Fresh, aerial parts of *Galeopsis pubescens* Besser (18 16 kg) were extracted in boiling EtOH 95% (2 × 10 l) After concn of the combined extracts in *vacuo*, H_2O was added and the aq soln was extracted with petrol The petrol-soluble part was rejected and the aq layer concd The aq concentrate was filtered through a prewashed (H_2O) neutral Al_2O_3 (5 kg) column eluting with H_2O The aq eluate was concd and lyophilized to give the crude glycoside fraction (375 g) A portion of the residue (164 g) was subjected to Craig distribution ($CHCl_3$–EtOH–H_2O, 3 2 5 [1]) and four fractions A–D were collected

Isolation of martynoside (**1**) *and isomartynoside* (**2**) The fraction A (1 19 g) was chromatographed on a reversed phase C-18 column (90 cm × 1 5 cm, i d) with MeOH–H_2O–*n*-PrOH (20 80 5, flow rate 2 ml/min) The faster eluate was concd in *vacuo* and the residue was lyophilized to give martynoside (**1**) (261 mg) as a yellowish amorphous powder, $[\alpha]_D^{20}$ −68 7° (*c* 0 690, MeOH) UV and IR data are similar as reported [2] ^{1}H NMR (CD_3OD) δ7 66 (1H, *d*, *J* = 15 9 Hz, H-β′), 7 20 (1H, *d*, *J* = 1 8 Hz, H-2‴), 7 08 (1H, *dd*, *J* = 1 8/8 3 Hz, H-6‴), 6 82 (1H, *d*, *J* = 8 3 Hz, H-5‴), 6 80 (1H, *d*, *J* = 8 1 Hz, H-5), 6 74 (1H, *d*, *J* = 2 0 Hz, H-2), 6 69 (1H, *dd*, *J* = 8 1/2 0 Hz, H-6), 6 37 (1H, *d*, *J* = 15 9 Hz, H-α′), 5 20 (1H, *d*, *J* = 1 7 Hz, H-1″), 4 92 (1H, *t*, *J* = 9 4 Hz, H-4′), 4 38 (1H, *d*, *J* = 7 9 Hz, H-1′), 3 89 and 3 82 (each 3H, *s*, 2 × Ar–OMe), 2 83 (2H, m, 2H-β), 1 10 (3H, *d*, *J* = 6 2 Hz, 3H-6″) The signals for 2H-α were overlapped with the sugar protons (4 06–3 28) ^{13}C NMR (see Table 1)

The slower running fractions gave isomartynoside (**2**) (242 mg) as a yellowish amorphous substance, $[\alpha]^{20}$ −45 7° (*c* 0 895, MeOH) EIMS 70 eV *m/z* $[M]^+$, 506 $[M-146\,(\text{rhamnosyl})]^+$, 476 $[M-176(\text{feruloyl})]^+$ 330 $[M-(146+176)]^+$, 168 [aglycone = 3-hydroxy-4-methoxy-phenylethylalcohol], 177 [feruloyl], 147 [terminal rhamnosyl] UV λ_{max}^{MeOH} nm 218 (4 37), 288 (4 18), 327 (4 31), IR ν_{max}^{KBr} cm^{-1} 3400, 1700, 1630, 1590 and 1510 ^{1}H NMR (CD_3OD) δ7 61 (1H, *d*, *J* = 16 0 Hz, H-β′), 7 13 (1H, *d*, *J* = 1 7 Hz, H-2‴), 7 06 (1H, *dd*, *J* = 1 7/8 3 Hz, H-6‴), 6 80 (1H, *d*, *J* = 8 3 Hz, H-5‴), 6 76 (1H, *d*, *J* = 8 2 Hz, H-5), 6 69 (1H, *d*, *J* = 2 1 Hz, H-2), 6 64 (1H, *dd*, *J* = 8 2/2 1 Hz, H-6), 6 37 (1H, *d*, *J* = 16 0 Hz, H-α′), 5 17 (1H, *d*, *J* = 1 0 Hz, H-1″), 4 33 (1H, *d*, *J* = 7 8 Hz, H-1′), 4 49/4 38 (each 1H, *m*, 2H-6′), 3 86 and 3 75 (each 3H, *s*, 2 × Ar–OMe), 2 80 (2H, *t*, *J* = 7 6 Hz, 2H-β), 1 25 (3H, *d*, *J* = 5 1 Hz, 3H-6″) The signals for 2H-α were overlapped with the sugar protons (4 02–3 29) ^{13}C NMR (CD_3OD) (see Table 1)

Acetylation of isomartynoside (**2**) Treatment of **2** (25 mg) with Ac_2O (1 ml) and pyridine (1 ml) at room temp overnight, followed by the usual work up afforded isomartynoside hepta-acetate (**3**) ^{1}H NMR ($CDCl_3$) δ7 66 (1H, *d*, *J* = 15 9 Hz, H-β′), 7 16–6 82 (6H, *m*, aromatic protons), 6 41 (1H, *d*, *J* = 15 9 Hz, H-α′), 4 80 (1H, *d*, *J* = 1 8 Hz, H-1″), 4 39 (1H, *d*, *J* = 7 9 Hz, H-1′), 4 18–4 20 (2H, *m*, 2H-6′), 4 08/3 62 (each 1H, *m*, 2H-α) 3 87 and 3 79 (each 3H, *s*, 2 × Ar–OMe), 2 80 (2H, *m*, 2H-β), 2 31 and 2 29 (6H, 2 × aromatic OAc), 2 12, 2 06, 2 05, 2 02 and 1 95 (15H, 5 × aliphatic OAc), 1 14 (1H, *d*, *J* = 6 2 Hz, 3H-6″)

Alkaline hydrolysis of isomartynoside (2) Compound 2 (50 mg) dissolved in 5 ml 5% KOH soln, was heated at 80° on an oil bath for 2 hr and then neutralized with 1 N HCl soln and evaporated to dryness The residue was chromatographed on a silica gel (40 g) column and eluted with EtOAc–MeOH–H_2O (100 16 5 13 5) The fractions were monitored on a silica gel prepared plate with CH_2Cl_2–MeOH–H_2O (80 20 2) The fractions containing the faster running substance were combined and evapd in *vacuo* to give ferulic acid (6 5 mg) ^{1}H NMR (CD_3OD) δ7 58 (1H, *d*, *J* = 15 9 Hz, Ar–CH=CH–), 7 16 (1H, *d*, $J_{2/6}$ = 1 9 Hz, H-2), 7 05 (1H, *dd*, $J_{6/5}$ = 8 2 Hz, $J_{6/2}$ = 1 9 Hz, H-6), 6 81 (1H, *d*, $J_{5/6}$ = 8 2 Hz, H-5), 6 30 (1H, *d*, *J* = 15 9 Hz, Ar–CH=CH–), 3 89 (3H, *s*, Ar–OMe) The fractions containing the slower running substance were evapd in *vacuo* and then lyophilized to give deacyl-glycoside 4 as a white amorphous powder (24 mg), $[\alpha]_D^{20}$ − 53 2° (*c* 0 524, MeOH) EIMS 70 eV *m/z* 476 ([M]$^+$, calc for $C_{21}H_{32}O_{12}$ 476 4826), 330 [M − rhamnosyl]$^+$, 168 [aglycone = 3-hydroxy-4-methoxy-β-phenylethylalcohol] (base peak), 152, 151, 150, 147 [terminal rhamnosyl] UV λ_{max}^{MeOH} nm 219 (3 94), 281 (3 58), ^{1}H NMR (CD_3OD) δ6 81 (1H, *d*, $J_{5/6}$ = 8 2 Hz, H-5), 6 73 (1H, *d*, $J_{2/6}$ = 2 0 Hz, H-2), 6 67 (1H, *dd*, $J_{6/5}$ = 8 2 Hz, $J_{6/2}$ = 2 0 Hz, H-6), 5 15 (1H, *d*, *J* = 1 6 Hz, H-1″), 4 29 (1H, *d*, *J* = 7 8 Hz, H-1′), 3 81 (3H, *s*, Ar–OMe), 2 81 (2H, *t*, *J* = 7 27 Hz, 2H-β), 1 25 (3H, *d*, *J* = 6 23 Hz, 3H-6″), 4 08–3 24 (*m*, 10H of glucose and rhamnose, 2H-α) ^{13}C NMR see Table 1

Acknowledgements—This work was supported by a reasearch grant of the Swiss National Science Foundation Thanks are due to Dr H Taguchi, Tsumura Laboratory, Tokyo, for an authentic sample of martynoside peracetate and Dr R K Chaudhuri, School of Pharmacy, University of Madison, Wisconsin, for preparing the manuscript

REFERENCES

1 Rogenmoser, E (1975) *Thesis* ETH Zürich, No 5574
2 Sasaki, H, Taguchi, H, Endo, T, Yosioka, I, Higashiyama, K and Otomasu, H (1978) *Chem Pharm Bull* **26**, 2111
3 Sticher, O, Salama, O, Chaudhuri, R K and Winkler, T (1982) *Helv Chim Acta* **65**, 1538
4 Sticher, O and Lahloub, M F (1982) *Planta Med* **46**, 145
5 Lahloub, M F (1983) *Thesis* ETH Zurich, No 7340