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IRIDOID GLUCOSIDES FROM *SATUREJA VULGARIS**

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Abstract—*Satureja vulgaris* was shown to contain two new iridoid glucosides, 5-deoxylamiol and 4-methylantirrhinoside, as well as the known iridoid glucosides lamiol and 5-deoxylamioside. The structures of the new glucosides were established by spectroscopic studies and chemical evidence

INTRODUCTION

Satureja vulgaris (L) Fritsch [2] is an herbaceous perennial plant, infusions, fluid and solid extracts of which are used in traditional medicine due to their stimulating and regulating action on the digestive apparatus [3] The pharmacological effects reported for *S vulgaris* and the presence in the extracts of a bitter constituent suggested to us the probable presence of iridoids in this plant

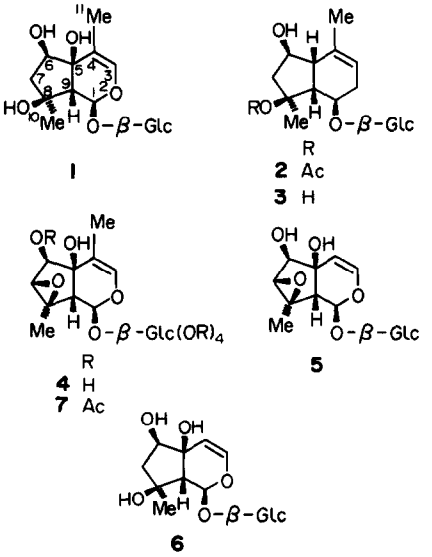
S vulgaris was extracted at room temperature with ethanol Paper chromatography of the extract showed the presence of four iridoids, which after the usual chromatographic purification followed by HPLC gave **1–4**

RESULTS AND DISCUSSION

Compounds **1** and **2** were shown to have identical properties to those of lamiol [4] and 5-deoxylamioside respectively Compounds **3** and **4** were new iridoids

Compound **3**, a white amorphous powder, R_f 0 47 (persistent pink spot with vanillin reagent) molecuar formula $C_{16}H_{26}O_9$, contained one glucose unit as demonstrated by acid hydrolysis Its ^1H NMR spectrum showed that both C-10 and C-11 carbons were at the oxidation level of methyl groups The whole signal pattern of the aglycone protons suggested a structure like 5-deoxylamiol and this was confirmed by the demonstration that alkaline hydrolysis in mild conditions of **2** [5] gave an iridoid which was identical to **3** and to a synthetic sample of 5-deoxylamiol (^1H NMR and IR superimposable [5]) It is important to note that compound **3** was shown by paper chromatography to be present in the ethanol extract, which was prepared under mild conditions, before purification This excluded any possibility that **3** was an artefact

Compound **4**, white amorphous powder, molecular formula $C_{16}H_{24}O_{10}$, $[\alpha]_D = -61°$, R_f 0 29 (dark violet with vanillin reagent), also contained one glucose unit as



demonstrated by acid hydrolysis Its ^1H NMR spectrum showed the presence of two methyl groups one at δ1 61 (J = 1, 5 Hz) attributed to a methyl group at C-4 coupled to vinylic proton H-3, and one at δ1 52 (*s*) attributed to a methyl group at C-8 geminal with an oxygen

The doublet (with integral value of one proton) at δ2 55 (J = 7 5 Hz) was assigned to H-9 coupled to H-1 and this established the presence of a hydroxyl function at C-5 The doublet at δ4 27 (J = 2 0 Hz) was attributed to H-6 geminal with a hydroxyl group coupled to H-7 which showed a doublet at δ3 61 (J = 2 0 Hz) It is important to note that the last value is peculiar to a proton geminal with an epoxide function located at C-7 and C-8 This was confirmed by Ross's test [6] which gave positive proof of the presence of an epoxide ring In addition, the $J_{6,7}$ value provided the key to the determination of the relative configuration at C-6 and C-7 as previously suggested by Rimpler *et al* [7] Indeed, the value 2 0 Hz is the same as that of antirrhinoside (**5**) [8] rather than of its C-6 epimer, procumbide [9], which does not show coupling, and

*Part 9 in the series "Iridoids in the Flora of Italy", for part 8 see ref [1]

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suggested that the stereochemistry of the cyclopentane ring of **4** should be the same as that of antirrhinoside (**5**)

PND and SFORD ^{13}C NMR spectra of **4** (see Table 1) confirmed the assignments of the aglycone moiety deduced by ^1H NMR analysis A doublet at δ6 6 9 and a singlet at 6 4 7 indicated an epoxide ring at C-7 and C-8 The doublets at δ7 6 0 and 5 3 9 and the singlet at 7 4 4 were assigned respectively to C-6, C-9 and C-5 The values at δ1 7 2 and 1 1 8 were easily assigned to two methyl groups at C-10 and C-11 respectively As regards the chemical shifts values of the cyclopentane ring they were comparable to those found to antirrhinoside (**5**) (see Table 1)

The doublet at δ1 3 7 7 and singlet at 1 1 5 2 were assigned at C-3 and C-4 respectively Of course these values were different from those of **5** owing to the presence of a methyl group at C-4 and in the comparison between ^{13}C NMR spectra of **4** and **5** (see Table 1) a paramagnetic shift for C-4 ($\Delta\delta = +7\,3$) and a diamagnetic shift for C-3 ($\Delta\delta = -5\,9$) were observed The same trend is shown by other C-4 methyl substituted/unsubstituted pairs of iridoids [10] These data therefore suggested for **4** the structure and configuration of 4-methylantirrhinoside

To verify this spectroscopic analysis **4** was (i) Acetylated under mild conditions to give the penta-acetylderivative **7** which, in its IR spectrum, showed a broad band of a free tertiary hydroxyl group (3400 cm^{-1}) which was sited at C-5 (ii) Treated with lithium in liquid ammonia, in these conditions reductive opening of the epoxide ring occurs, to give lamiol (**1**) as main product This proved that the chiral centres C-1, C-5, C-6, C-8 and C-9 had the same absolute configuration in **4** and **1** Consequently the epoxide ring in **4** had to be identical to the one in antirrhinoside (**5**), i e in the β-configuration

All the reported data showed that **4** was 4-methyl-antirrhinoside

EXPERIMENTAL

CC silica gel 70–230 mesh and cellulose CF 11, TLC silica gel SIF_{254} and cellulose plates, PC Schleicher & Schull n° 2043 b

Table 1 ^{13}C NMR (20 MHz, D_2O, dioxane (67 4 ppm from TMS) as int standard)

C	4	5	1	6
1	95 4	94 9	93 1	93 6
3	137 7	142 9	136 1	142 0
4	115 2	107 5	114 6	107 3
5	74 4	74 3	72 6	71 3
6	76 0*	76 8*	73 9*	77 1*
7	66 9	66 2	46 8	46 2
8	64 7	65 0	75 8	77 8
9	53 9	52 1	58 9	57 8
10	17 2	17 0	23 8	24 7
11	11 8	—	11 9	—
1′	99 0	99 2	98 7	99 0
2′	73 5	73 5	73 3*	73 3
3′	76 5*	76 4*	76 5†	76 2*
4′	70 4	70 4	70 5	70 5
5′	77 1*	77 1*	77 0†	77 1*
6′	61 6	61 6	61 5	61 5

*,† Values with the same superscript in the vertical column are interchangeable

Mgl paper Spray reagents 2N H_2SO_4, vanillin (vanillin 2 g, conc HCl 4 ml, MeOH 100 ml) and resorcin (resorcin 5 g, conc H_2SO_4 4 ml, EtOH 300 ml) All evaporations of volatile material were performed under red pres

Isolation of iridoid-containing fractions Flowering plants of *S vulgaris* (= *Clinopodium vulgare* L) were collected in June 1982 in the neighbourhood of Vicovaro (Roma, Italy) Voucher specimens of the plant were identified by Dr Anna Francesconi, Istituto di Botanica dell'Università di Roma

Fresh aerial parts of the plant (4 kg) were extracted with 90% EtOH (8 l × 2) at room temp for 3 days PC in *n*-BuOH–HOAc–H_2O (63 10 27) showed the presence of four iridoids with R_f values of 0 59 (5-deoxylamioside, **2**), 0 47 (5-deoxylamiol, **3**), 0 37 (lamiol, **1**) and 0 29 (4-methylantirrhinoside, **4**) The ethanolic extract was concd to an aq suspension which was treated with decolourizing charcoal (750 g) The resulting suspension was stratified on a Gooch funnel (20 cm ϕ) Monosaccharides were eluted with H_2O (10 l), oligosaccharides with 5% (5 l) and 10% (5 l) EtOH, **1**, **4** and small quantities of **2** and **3** with 30% EtOH (4 l), **2**, **3** and small quantities of **1** and **4** with 50% (3 l) and 80% (3 l) EtOH

The 30% EtOH fraction (4 5 g) was chromatographed on cellulose in *n*-BuOH sat H_2O to give in the first fractions crude **2** and **3** (350 mg) and then crude **1** (100 mg) and **4** (600 mg) The 50% and 80% EtOH fractions (2 5 g) were chromatographed on cellulose in *n*-BuOH sat H_2O giving 850 mg crude **2** and **3** and successively 10 mg crude **1** and 50 mg crude **4** Fractions containing crude **1** (110 mg) were purified on silica gel in $CHCl_3$–MeOH (7 3) affording 50 mg **1** which on HPLC on a semipreparative μ-Bondapak C_{18} column (Waters, ϕ 4 in, grain size 10 μ) eluted with H_2O–MeOH (7 3, 3 ml/min, UV 210 nm) gave 40 mg **1** identical to an authentic sample of lamiol (^1H NMR and IR superimposable)

Fractions containing crude **2** and **3** (1 2 g) were chromatographed on silica gel in $CHCl_3$–MeOH (3 1) giving in the first fractions **2** (300 mg) and then **3** (450 mg) Compound **2** was purified by HPLC, conditions as just described except for use of H_2O–MeOH (1 1), to give 250 mg of pure **2** identical to an authentic sample of 5-deoxylamioside (^1H NMR and IR superimposable) Compound **3** was purified by HPLC in the same way as **2** to give 400 mg **3** ^1H NMR 90 MHz (D_2O) δ6 05 (H-3, *m*), 5 45 (H-1, *s*, *br*), 2 60 (H-5 and H-9), 2 10 and 1 87 (2H-7, AB part of an ABX system, $J_{AB} = 14\,0$, $J_{A,6} = 7\,0$, $J_{B,6} = 6\,0$ Hz), 1 64 (3H-11, *s*, *br*), 1 30 (3H-10, *s*) [Calc for $C_{16}H_{26}O_9$ C 53 03, H 7 23 Found C 52 94, H 7 30%] Compound **3** proved to be identical to a synthetic sample of 5-deoxylamiol

Fractions containing crude **4** (650 mg) were purified on silica gel in $CHCl_3$–MeOH (7 3) to give 500 mg **4** which on HPLC under the same conditions as those used for **1** gave 450 mg **4** as an amorphous powder $[\alpha]_D^{25} = -61°$ (MeOH, *c* 0 1), IR ν_{max}^{KBr} cm^{-1} 3450, 2950, 1670, 1390, 1110, 1090, 1070 and 1000, ^1H NMR 90 MHz (D_2O) δ6 21 (H-3, *q*, $J_{3\,11} = 1\,5$ Hz), 5 30 (H-1, *d*, $J_{1\,9} = 7\,5$ Hz), 4 75 (H-1′, *d*, $J_{1\,2} = 7\,5$ Hz), 4 27 (H-6, *d*, $J_{6\,7} = 2\,0$ Hz), 3 61 (H-7, *d*, $J_{7\,6} = 2\,0$ Hz), 2 55 (H-9, *d*, $J_{9\,1} = 7\,5$ Hz), 1 61 (3H-11, *d*, $J_{11,3} = 1\,5$ Hz), 1 52 (3H-10, *s*) [Calc for $C_{16}H_{24}O_{10}$ C 51 06, H 6 43 Found C 50 93, H 6 50%]

Penta-O-acetyl derivative of **4** (**7**) Compound **4** (100 mg) was treated with dry pyridine (0 5 ml) and Ac_2O (1 0 ml) for 1 5 hr at room temp After addition of MeOH (3 ml), the soln was left for 20 min, then evaporated to give crude **7** (110 mg) which was chromatographed on silica gel in C_6H_6–Me, *t*-Bu ether (1 1) to give 90 mg **7** which crystallized from EtOH as prisms, mp 155–156° ^1H NMR 90 MHz ($CDCl_3$) δ6 08 (H-3, *q*, $J_{3\,11} = 1\,5$ Hz), 5 15 (H-1, *d*, $J_{1\,9} = 8\,0$ Hz), 5 10 (H-6, *d*, $J_{6\,7} = 1\,5$ Hz), 4 24 (2H-6′, *m*), 3 70 (H-5′, *m*), 3 55 (H-7, *d*, $J_{7\,6} = 1\,5$ Hz), 3 20 (OH-5), 2 45 (H-9, *d*, $J_{9\,1} = 8\,0$ Hz), 2 18, 2 08

and 2 00 (acetyls, 1 1 3), 1 55 (3H-11, *d*, $J_{11\,3}$ = 1 5 Hz), 1 50 (3H-10, *s*)

Li/NH_3 *reduction of* **4** **4** (200 mg) was dissolved in liquid NH_3 (60 ml), the soln was cooled to −40° and 100 mg Li added, after 20 min, 0 5 ml EtOH were added At intervals of 15 min both the additions were repeated × 3 The reaction was stopped after 2 hr by addition of EtOH (5 ml) and the mixture was left overnight at room temp The residue was treated with H_2O (50 ml), neutralized with CO_2 and the EtOH removed *in vacuo*

The resulting soln was treated with decolourizing charcoal (4 g) and the mixture stratified on a Gooch funnel (1 cm i d), washed with H_2O until the washings gave a negative salt test and then eluted with MeOH (100 ml) The MeOH soln gave a residue (150 mg) which was chromatographed on silica gel in $CHCl_3$–MeOH (7 3) giving in the first fractions unreacted **4** (40 mg) and then **1** (40 mg) Compound **1** was identified by comparison with an authentic sample of lamiol (^{1}H NMR and IR superimposable)

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