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LUTEOLIN 5-RUTINOSIDE FROM *SALVIA LAVANDULIFOLIA* SSP. *OXYODON*

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Key Words Index—*Salvia lavandulifolia* ssp. *oxyodon*; Lamiaceae; luteolin 5-*O*-β-rutinoside.

Abstract—The flavonoid, luteolin 5-rutinoside, has been isolated from *Salvia lavandulifolia* ssp. *oxyodon* and identified by enzymic hydrolysis and spectroscopic methods.

INTRODUCTION

Various species of the genus *Salvia* are used as folk remedies to treat several disease states, most notably diabetic hyperglycemia. *Salvia lavandulifolia* Vahl. ssp. *oxyodon* (Webb & Heldr) [1], a species which grows exclusively within Spanish borders (particularly in the province of Granada), has been previously studied for its utility in the treatment of diabetes mellitus. Thus, Delia [2] and Ferranini [3] have reported the hypoglycemic and antidiabetic activity of a methanolic extract of this crude drug in diabetic subjects. Jiménez *et al.* [4] described the hypoglycemic and antidiabetic activities of infusions and suspensions of *S. lavandulifolia* on normoglycemic and alloxan-diabetic rabbits. More recently Zarzuelo *et al.* [5] have demonstrated the hypoglycaemic activity of an aqueous extract of its aerial parts, as well as the mechanism of its hypoglycaemic action.

The present study was undertaken to isolate and identify a new flavonoid (**1**) from *S. lavandulifolia*.

RESULTS AND DISCUSSION

The major compound **1** was obtained by polyamide column chromatography, and showed a major peak on HPLC (T_R = 2.6 min), whose UV spectrum had two bands characteristic of a flavone glycoside. The colour of the glycoside appeared blue when spotted on polyamide and viewed under UV light (366 nm), and remained blue when exposed to NH_3 vapour. These results indicated that the 5-hydroxyl group was substituted. Enzymic hydrolysis of **1** with β-glucosidase (pH = 5 and 37°) yielded luteolin, which was identified by HPLC by comparison with a pure standard. Elemental analysis of the isolated compound from *S. lavandulifolia* found: C, 55.0; H, 5.6. $C_{27}H_{30}O_{15}$ requires: C, 54.5; H, 5.05%, confirmed the identification of the active principle as luteolin 5-*O*-β-rutinoside. Two anomeric proton signals at δ 5.1 (1H, *d*, J = 7 Hz, Glu H-1) and δ 4.15 (1-H, *s*, Rha H-1), a three proton singlet for the methyl of the rhamnose at 0.88 in ^{1}H NMR and the downfield displacement in the ^{13}C NMR signal of glucose C-6 (δ 67.5), suggested the presence of rutinose. The bathochromic shift of band I and band II with $AlCl_3$, and a hypsochromic shift with $AlCl_3$/HCl indicated that the 5-hydroxyl group is substituted and the presence of an *ortho*-dihydroxy B-ring. The substituted 5-hydroxyl was confirmed by the ^{1}H NMR spectrum data (singlet at δ 6.6 of H-3 between two doublets at δ 6.4 of H-6 and δ 6.7 of H-8) and the ^{13}C NMR displacement of C-5 (δ 144.0). The bathochromic shift of band I with NaOMe without any decrease in intensity showed the presence of a free hydroxyl group at C-4′. In addition, the presence of a free hydroxyl group at C-7 was demonstrated by the bathochromic shift of band II in the spectrum with NaOAc. Both the NaOAc H_3BO_3 and $AlCl_3$ spectra indicated the presence of an *ortho*-dihydroxyl group in the B ring [6].

From these data **1** was characterised as luteolin 5-*O*-rutinoside, which is reported here for the first time from *Salvia* and in nature. However, different luteolin glycosides have been described previously from other subspecies of *S. lavandulifolia* and 5-glycosylated flavones have been reported from other *Salvia* species, for example 6-hydroxyluteolin 5-glucoside [7] and salvigenin 5-glucoside [8] from *S. tomentosa* and *S. virgata*, respectively.

EXPERIMENTAL

Plant material. The aerial parts of *S. lavandulifolia* ssp. *oxyodon* were collected in Sierra Nevada (province of

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Granada, Spain) at an altitude of approximately 2000 m above the sea level and identified by Dr Socorro (Department of Botany, University of Granada, Spain). A voucher specimen was deposited at the Herbarium of the Department of Botany, University of Granada, Spain (ref. no. 19791). The samples were warm air-dried, powdered and stored at 5 until use.

Extraction and isolation. The powdered aerial parts (500 g) were extracted using a soxhlet with a series of solvents of increasing polarity (hexane, $CHCl_3$, EtOAc, EtOH and MeOH). The methanolic extract was concentrated to dryness (yield 3.9%) under reduced press. and the dry residue subjected to mid-pressure chromatography (LABOMATIC-2000, 4 bar, 5 ml min^{-1}) using a column (30 × 2 cm) with polyamide (Macherey-Nagel, CC6, 0.05–0.16 mm) as stationary phase and MeOH–H_2O mixtures as mobile phase [8]. All collected fractions were subjected to silica gel TLC and HPLC. The TLC solvent used was Me_2CO–$CHCl_3$–MeOH–H_2O (6:1:2:1). HPLC was carried out using a Waters chromatograph with a Perkin-Elmer L-135 diode array detector, a Spherisorb RP-18 5μ (25 × 250 mm) column and MeOH–H_2O (3:2) as mobile phase at a flow rate of 1 ml/min.

CC yielded 250 fractions (10 ml each). Compound **1** was found in fraction 107–140. These fractions were combined, the solvent evaporated under low pressure and the dry residue rechromatographed by identical procedures as those previously described.

Compound **1** was identified by (*a*) *elemental analysis*, performed in a Perkin-Elmer 240-C sample analyser; (*b*) *enzymic hydrolysis*: the glycoside was dissolved in sodium acetate buffer 0.5 M to (pH = 5) and small amount of β-glucosidase (Sigma Corp.) was added. The mixture was allowed to stand overnight at 37 and the resultant solution was concentrated under high vacuum [6]. Luteolin 5-rutinoside should not be hydrolysed by β-glucosidase but the breakdown to aglycone may be due to the overnight treatment at pH 5 and 37 . (*c*) *UV spectrometry and* ^1H *NMR and* ^{13}C *NMR.*

^1H *NMR spectral data for* **1**. (300 MHz) DMSO (δ):0.88 (3H, *s*, Rha CH_3-6), 3.15 (2H, *s*, Glu CH_2-6), 3.2–3.8 (*m*, sugar protons), 4.15 (1H, *br s*, Rha H-1), 5.1 (1H, *d*, J = 7 Hz, Glu H-1), 6.42 (1H, *d*, J = 1.9 Hz, H-6), 6.61 (1H, *s*, H-3), 6.78 (1H, *d*, J = 1.9 Hz, H-8), 6.92 (1H, *d*, J = 7.8 Hz, H-5′), 7.42 (1H, *d*, J = 7.8 Hz, H-6′), 7.44 (1H, *s*, H-2′).

^{13}C *NMR spectral data for* **1**. (300 MHz) DMSO (δ):18.7 (Rha C-6), 67.5 (Glu C-6), 69.4 (Rha C-5), 73 (Rha C-2, C-3), 73.1 (Glu C-4), 76.3 (Glu C-2), 76.3 (Rha C-4), 77.0 (Glu C-3, C-5), 94.6 (C-8), 99.4 (Rha C-1), 99.8 (Glu C-1), 104.0 (C-3), 105.2 (C-10), 113.5 (C-6), 115.4 (C-5′), 115.9 (C-2′), 119.0 (C-6′), 121.2 (C-1′), 144.0 (C-5), 145.7 (C-3′), 149.8 (C-4′), 156.8 (C-9), 162.8 (C-2), 164.4 (C-7), 181.1 (C-4).

UV spectral data for **1**. λ_{max}^{MeOH} nm: 258, 272 sh, 348, + NaOMe: 268, + NaOAc: 264, 372, + NaOAc/H_3BO_3: 262, 370, + $AlCl_3$: 268, 424, + $AlCl_3$/HCl: 260, 348.

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