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A new acetylated glucoside of luteolin and two flavone glucosides from *Lavandula stoechas* ssp. *stoechas*

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Received December 2, 2002, accepted January 12, 2003

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Pharmazie 58: 426–427 (2003)

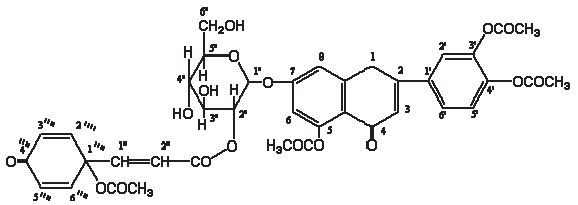
Fractionation of ethyl acetate extract of *Lavandula stoechas* aerial parts revealed the presence of a novel acetylated glucoside (**1**) together with apigenin 7-*O*-glucoside (**2**) and luteolin 7-*O*-glucoside (**3**). The structures of these compounds were elucidated by spectroscopic analyses, notably UV, MS, and NMR.

1. Introduction

Despite of the widespread use of the essential oil of *Lavandula stoechas* [1], little work has been done to characterize *L. stoechas* phenolic compounds. 50 species of the genus *Lavandula* including *L. stoechas* are used in several phytotherapeutic preparations [2] mainly because the suspension and infusion of *L. stoechas* shows hypoglycemic activity. Decoctions and infusions of leaves and inflorescence of *L. stoechas* are currently used in Crete by traditional therapists [3] against diabetes, hypertention, neuralgia and as spasmolytic. In this study on the phenolic compounds of *L. stoechas* we wish to report the isolation and structure elucidation of a new acetylated glucoside together with apigenin 7-*O*-glucoside and luteolin 7-*O*-glucoside.

2. Investigations, results and discussion

A methanol extract of the aerial parts of *Lavandula stoechas* was suspended in water and fractionated into diethyl ether, ethyl acetate and *n*-butanol soluble fractions. Repeated column chromatography of the ethyl acetate soluble fraction afforded three flavone glucosides. The spectroscopic data of **2** and **3** were in excellent agreement with those reported for apigenin 7-*O*-glucoside and luteolin 7-*O*-glucoside. The constituent **1** seems to be a new compound and was determined as follows.



Compound **1** was assigned the molecular formula $C_{38}H_{34}O_{18}$ on the basis of the FAB-MS (positive ion mode) spectrum at *m/z* 779 $[M + H]^+$. The ^1H NMR spectrum of **1** showed most of the characteristics of a 7-*O*-substituted luteolin, with a one proton singlet at δ 6.42 (H-3), two one proton doublets (J = 2 Hz) at δ 6.55 and 6.90 (H-6 and H-8), a one proton doublet (J = 2.5 Hz) at δ 7.13 (H-2′), a one proton doublet (J = 9 Hz) at 7.32 (H-5′) and a one proton doublet of doublets (J = 2.5 and 9 Hz) at 7.24 (H-6′). The appearance of the olefinic protons (H-α and H-β) at 6.32 and 6.72 ppm (J = 16 Hz) suggested the presence of a trans *p*-coumaroyl moiety. Since H-1‴ and H-2‴ was shifted upfield (relative to those in 2 [4]) and characteristic signals for a conjugated ketone (IR V_{max} cm^{-1}) were observed, a different acyl moiety was suggested [5]. The anomeric proton at 5.00 ppm indicated the presence of a β-glucosyl moiety. The coupling constant of H-1′ (J = 7.5 Hz) indicated β configuration of the sugar moiety. Also the ^1H NMR spectrum exhibited a signal at δ 2.5 due to four aromatic acetyls. Furthermore the appearance of a signal at 5.38, dd (j = j′ = 9 Hz) suggested the attachement of the propenoic ester in the C-2 position of the sugar [6]. The FAB-MS of the parent ion peak (*m/z* 573) demonstrated the presence of an acylated flavonoid with a glucose moiety. The fragment at 413 indicated the flavonoid moiety, while fragments at 371 and 329 indicated stepwise loss of 42 amu from *m/z* 413. The EIMS showed the characteristic fragment peaks due to the acylated flavonoid: *m/z* 412 $[M^+]$, 383 $[M^+$-CHO], 369 [M-CH_3COH], 355 $[M^+$-CHO–CO], 313 [355-CH_3CO], 284 [313-COH], 256 [284-CO], 221$[B_1^+]$, 194 $[A^+]$, 165 $[A^+$-129]. Also there are the following fragments due to the coumaroyl ketone 222 [O=C_6H_4(OCOCH_3)CH=CHCOOH], 194 [222-CO], 152 [O=C_6H_4(OCOCH_3)H], 135 [O=C_6H_4(OH)CH=CH], 124 [152-CO], 109 [124-CH_3]. The presence and the linkage of glucose moiety was also established and confirmed by acidic, alkaline and enzymatic hydrolysis.

3. Experimental

3.1. General

Melting points were obtained on an Electrothermal 9300 apparatus and are uncorrected. UV spectra were recorded on a Hitachi 2000 spectrometer. Electron impact mass spectra (EIMS) were obtained by direct-inlet solid-probe analysis using a VG-Trio 2000 spectrometer at 70 eV. FAB-MS

spectra were taken on a DG/600 spectrometer with 3-nitro-benzyl alcohol as matrix. IR spectra were recorded on a Perkin-Elmer 567 apparatus. ^{1}H NMR experiments were recorded on a Varian VXR 400 using TMS as internal standard.

3.2. Plant material

Aerial parts of Lavandula stoechas were collected in Chalkidiki, Greece, in 1998. A voucher specimen has been deposited at the herbarium of Systematic Botany No (44).

3.3. Extraction and isolation

Air-dried aerial parts of the plant (500 g) were exhaustively extracted (Soxhlet) with petrol (b.p. 50–70 °C), CH_2Cl_2 and MeOH. The later extract was concentrated and the residue redissolved in boiling H_2O. The H_2O soluble fraction was filtered and extracted successively with Et_2O, EtOAc and *n*-BuOH. The ETOAc extract was concentrated under reduced pressure to yield a ppt. (4 g) 1 g of which was repeatedly chromatographed in Polyamide MNCC6 column (30 × 5) cm with a H_2O–MeOH gradient elution system to give compound **1** from 50–60% MeOH; **2** from 20–30% MeOH and **3** from 40–50% MeOH. After final purification on a Sephadex LH-20 column with MeOH gave 10 mg of **1**, 6 mg of **2** and 5 mg of **3**.
Apigenin 7-*O*-glucoside and luteolin 7-*O*-glucoside were identified by m.ps, UV, ^{1}H NMR and EIMS spectral data.

3.3.1. 5,3′,4′-Triacetylated-luteolin-7-O-glucoside 2″-(4‴″-oxa 1‴″-acetylo 2‴″,5‴″ cyclohexadiene) 2‴-propenoic ester (**1**)

Mp. 213–214 °C uncorr., TLC (Cellulose): Rf 0.90 BAW (BuOH-acetic acid-H_2O, org. phase 4 : 1 : 5), 0.04 (HOAc 15%), 0.85 EAW (AcOEt-HOAc-H_2O, org phase 8 : 2 : 4) Spot appearance durk (UV), durk f_1 (UV/NH_3) UV(λ_{max}, MeOH nm): 253, 300 IR: (KBr) cm^{-1} : 3360 (OH), 1710 (ester C=O), 1666 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 7.49 (2 H, d, J = 9.5 Hz, H-3‴″, H-5‴″), 7.32 (1 H, d, J = 9.0 Hz, H-5′), 7.24 (1 H, dd; J = 9.0, 2.5 Hz, H-6′), 7.13 (1 H, d, J = 2.5 Hz, H-2′), 7.07 (2 H, dd, J = 9.5, 1.8 Hz, H-2‴″, H-6‴″), 6.90 (1 H, d, J = 2 Hz, H-8), 6.70 (1 H, d, J = 16 Hz, H-1‴), 6.55 (1 H, d, J = 2 Hz, H-6), 6.42 (1H, s, H-3), 6.35 (1 H, d, J = 16 Hz, H-2‴), 5.38 (2 H, dd, j = j′ = 9 Hz, H-2″), 5.00 (1 H, d, J = 7.5 Hz, H-1″). FAB MS, positive ion mode, (rel. int.) *m/z*: 779 $[M + H]^+$ (4), 573 [flavonoid + glucose] (6), 413 [flavonoid] (25), 371 [413-CH_2CO] (10), 329 [371-CH_2CO] (17), 176 [O=C_6H_4(OCOCH$_3$)–CH=CH–H^+] (100), EIMS 70 eV, *m/z* (rel. int.): 412 $[M^+]$ (5), 383 $[M^+ -29]$ (3), 369 $[M^+ -43]$ (5), 355 $[M^+ -29–28]$ (2), 313 [355–42] (6), 284 [313–29] (10), 256 [284–28] (16), 221 $[B_1^+]$ (5), 222 [O=C_6H_4(OCOCH$_3$)CH=CHCOOH] (10), 195 $[A^+]$ (7), 165 $[A^+ -29]$ (10), 152 [O=C_6H_4(OCOCH$_3$)H] (13), 124 [152–28] (25), 109 [124–15] (60).

3.3.2. Acidic hydrolysis

A small amount of **1** was refluxed with 2M HCl and yielded two products which on TLC (cellulose) gave two spots. Rf values: 87 and 28 (BAW), 08 and 04 (HOAc 15%). These products were isolated by PTLC (Cellulose, EAW) and their structures were proved as 7-OH, 5,3′,4′ triacetylo luteolin and glucose 2″(4‴″-oxa 1‴″-acetylo 2‴″,5‴″-cyclohexadiene) 2‴propenoic ester by EIMS (VG, Trio2000, 70 eV) with the following characteristic fragments: First spot 412 M^+; 384 $[M^+ -28]$; 218 $[B_1^+]$, 195 $[A_1^+]$. Second spot 222$[M^+]$, 177 [O=C_6H_4 (OCOCH$_3$) CH=CH], 134 [O=C_6H_4(OH) C≡CH].

3.3.3 Alkaline hydrolysis

Constituent **1** was treated with 2 M NaOH at room temperature for 30 min. After acidification with 2 M HCL and evaporation the residue was taken into boiling water and extracted with EtOAc. TLC (cellulose) of the EtOAc extract gave two spots with Rf values 45 (BAW) 18 (HOAc 15%) for the first spot and 90 (BAW), 50 (HOAc 15%) for the second. Enzymatic cleavage of the first spot with β-glucosidase yielded luteolin and β-D-Glucose (co-chromatography with authentic sample)

Acknowledgements: The authors wish to thank the Department of Chemistry, University of London for the ^{1}H NMR spectra and FAB-MS and the Mediterranean Agronomic Institute for EIMS facilities.

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