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THREE METHYLATED FLAVONES FROM *THYMUS VULGARIS*

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Key Word Index—*Thymus vulgaris*; Labiatae; new flavones; 5,6,4′-trihydroxy-7,8,3′-trimethoxyflavone; 5,4′-dihydroxy-6,7,3′-trimethoxyflavone; cirsilineol; 5,4′-dihydroxy-6,7,8,3′-tetramethoxyflavone.

Abstract—Three highly oxygenated flavones were isolated from leaves of *Thymus vulgaris*. Their structures were determined by spectroscopic methods as 5, 6, 4′-trihydroxy-7, 8, 3′-trimethoxyflavone (thymonin), 5, 4′-dihydroxy-6, 7, 3′-trimethoxyflavone (cirsilineol) and 5, 4′-dihydroxy-6, 7, 8, 3′-tetramethoxyflavone. These flavones are reported for the first time in the genus *Thymus*.

INTRODUCTION

We previously reported[1] that the essential oil in thyme extracts could not be responsible for their spasmolytic action. While seeking the active components of these preparations, biological screening of different fractions, obtained by CC, yielded the new flavonoid 5, 6, 4′-trihydroxy-7, 8, 3′-trimethoxyflavone (**2**), called thymonin, 5, 4′-dihydroxy-6, 7, 8, 3′-tetramethoxyflavone (**3**) and 5, 4′-dihydroxy-6, 7, 3′-trimethoxyflavone (**1**). **3** is already reported in the genus *Sideritis*[2, 3]; and **1** (cirsilineol) in the genera *Sideritis*[2], *Artemisia*[5, 7], *Anisomeles*[6] and *Salvia*[4]. Up to now the only flavonoids described in *Thymus vulgaris* L. are luteolin, luteolin-7-β-glucoside, luteolin-7-diglucoside[8], 6-hydroxy-luteolin[9], apigenin, naringenin and the Stoess' compound J[10]. **1–3** are reported for the first time in the genus *Thymus*.

RESULTS AND DISCUSSION

Ground, dried leaves of *T. vulgaris* were extracted with aqueous methanol. The aqueous layer, obtained after removal of the methanol, was extracted consecutively with hexane and chloroform. CC of the concentrated chloroform extract afforded cirsilineol (**1**), thymonin (**2**) and 5, 4′-dihydroxy-6, 7, 8, 3′-tetramethoxyflavone (**3**). UV spectra, carried out with diagnostic reagents using standard procedures[11], as well as ^{1}H and ^{13}C NMR revealed a great similarity between **1**, **2** and **3**. The absence of any signal other than those of the methoxyl groups (singlets around δ 3.7–4) below δ 5 in the ^{1}H NMR spectra excluded the presence of the flavanone of dihydroflavonol structure, leaving flavones and flavonols as the only possibilities. These findings were confirmed by the UV spectra in methanol which were typical for flavones or flavonols having hydroxyl or methoxyl substituents at both 3′ and 4′ positions[11, 12]. The absence of the H-3 singlet, usually present in the ^{1}H NMR spectrum at *ca* δ 6.3 in TMSi derivatives indicated that there were flavones. It has been reported frequently[5, 11] that this signal is shifted to *ca* δ 6.9 when DMSO-d_6 is used as solvent. The ^{13}C NMR spectrum exhibited signals at δ 163,7 and 102.7 for C-2 and C-3 respectively, which corresponded to a flavone structure[15, 16]. Comparison of the peak patterns at *ca* δ 7 and 7.6 with many published ^{1}H NMR spectra of flavonoids also suggestd 3′, 4′-disubstitution[1], confirmed by a ^{13}C-^{1}H gated decoupling experiment. The best correlation with previously published data is with those for 3′-methoxy-4′-hydroxy analogues[13]. The set of six signals corresponding to the carbon atoms 1′–6′ are found at identical values in **1–3** proving an identical B-ring substitution. Addition of sodium methoxide to the methanolic solution showed bathochromic shifts in the UV spectra of 57 nm (**1**), 48 nm (**2**) and 65 nm (**3**), without decrease in absorbance, which is indicative of a 4′-hydroxyl group[11]. Shifts for band I in methanol (I + 21 nm; II, + 25 nm; III, + 21 nm) suggested presence of a hydroxyl at position 5. The relative low bathochromic shift is characteristic of flavones and 3-*o*-substituted flavonols which contain a methoxyl or hydroxyl at C-6[14]. The rather high chemical shift value of the carbonyl carbon at position 4 confirmed the presence of the hydroxyl group at position 5[18, 16]. Addition of sodium acetate–boric acid to the methanolic solutions did not affect the UV spectra, indicating the absence of *o*-hydroxy groups in both of the rings[11, 17]. The structural differences between the three 5, 4′-dihydroxy-3′-methoxyflavones were therefore located in the A-ring at positions 6–8.

The MS of **1** exhibited a molecular ion peak at *m/z* 344 (98%) in accord with a flavone containing two hydroxyls and three methoxyls. UV data established

the presence of a methoxyl at position 6. Chemical shifts of the carbon atoms of the A-ring correlated perfectly with those of meliscinplin[13], having a 5-hydroxy-6, 7-dimethoxy A-ring substitution and differed substantially from the shifts of the 5-hydroxy-7, 8-dimethoxy isomer (ternatin). Since UV and NMR spectra of eupatorin[18, 19] also showed other data, **1** has to be 5, 4'-dihydroxy-6, 7, 3'-trimethoxyflavone (cirsilineol). This was in accordance with data described in ref. [5].

The molecular ion peak of **2** was at *m/z* 360 (68%), suggesting that it contained one hydroxyl more than **1** in the A-ring. Location of the hydroxyl group was not possible from NMR data for sterically crowded molecules[13]. Treatment with *p*-benzoquinone did not result in the formation of a red colour or of a precipitate (gossypetone reaction), thereby eliminating the possibility of the second hydroxyl group at position 8[20]. UV data indicated methylation of the hydroxyl at position 7. From these results, 5, 6, 4'-trihydroxyl-7, 8, 3'-trimethoxyflavone is the only possible structure for **2**.

The MS of **3** gave a molecular ion peak at *m/z* 374 (77%) in accord with a flavone containing two hydroxyls and four methoxyls. Since one methoxyl and two hydroxyls were already determined, the three other methoxyl groups must be assigned to the only available positions C-6–C-8, leading to 5, 4'-dihydroxy-6, 7, 8, 3'-tetramethoxyflavone as the structure for **3**. UV, IR and NMR data were in good agreement with those reported by Rodriguez[2].

EXPERIMENTAL

Plant material. The aerial parts of *T. vulgaris* cultivated in Leuven (Belgium) were collected in August 1980.

General techniques. CC employed Si gel 60 (E. Merck) and Sephadex LH-20 (Pharmacia). Precoated plates: Si gel GF-254 (E. Merck) and Polyamide 11 F-254 were used for TLC. The solvent systems were: A (toluene–EtOAc–HCO_2H, 58:33:9); B (C_6H_6–C_5H_5N–HCO_2H, 72:18:10); C (toluene–$CHCl_3$–Me_2CO–HCO_2H, 40:30:25:5); D ($CHCl_3$–MeOH, 90:10); E [C_6H_6–petrol (bp. 60–80°)MeCOEt–MeOH, 60:26:7:7] and F (C_6H_6–MeCOEt–MeOH, 4:3:3). Prep. TLC: precoated Si gel plates (1 mm), solvent system B. The yellow bands were eluted with MeOH. Flavonoids were visualized either by UV light + NH_3 or by spraying with NA (Naturstoffreagenz-A) in MeOH.

Isolation of flavonoids. Ground, dried leaves were extracted with 85% aq. MeOH and 50% aq. MeOH. The combined extracts were evaporated under red. pres. until only H_2O remained. The ppt obtained on standing in the cold was removed and the aq. layer partitioned with *n*-hexane and $CHCl_3$. The concd $CHCl_3$ extract was chromatographed over a Si gel column, packed in $CHCl_3$, using a mixture of $CHCl_3$–MeOH with an increasing ratio of MeOH. Fractions containing **1–3**, detected by TLC, were collected, concd and purified over a Sephadex LH-20 column, eluted with EtOH. Total purification was obtained by prep. TLC.

Identification of flavonoids. For ^{13}C NMR, see Table 1. Cirsilineol (**1**): R_f values: A, 0.40; B, 0.53; C, 0.36; D, 0.64; E, 0.28; F, 0.82. ^1H NMR (100 MHz, DMSO-d_6): δ 3.76 (3H, *s*, -OMe), 3.92 (3H, *s*, -OMe), 3.96 (3H, *s*, -OMe), 6.82–7.08 (3H, *m*, H-3, H-5' and H-8), 7.60–7.78 (2H, *m*, H-2' and H-6'). EIMS 70 eV, *m/z* (rel. int.) 344 $[M]^+$ (98) for $C_{18}H_{16}O_7$, calc. 344.0896; found, 344.0897, 343 $[M-H]^+$ (21), 339 $[M-Me]^+$ (100), 315 $[M-COH]^+$ (22), 301 $[M-MeCO]^+$ (28), 181 $[A_1-Me]^+$ (21), 153 $[A_1-MeCO]^+$ (42), 151 $[B_2]^+$ (9) and 148 $[B_1]^+$ (3). A_1, B_1 and B_2 terminology for fragments is given in ref. [12]. UV λ_{max} nm: (MeOH) 241, 252sh, 275, 343; (NaOMe) 266, 401; ($AlCl_3$) 240sh, 262, 284, 300sh, 378; ($AlCl_3$–HCl) 235sh, 259, 289, 365; (NaOAC) 267, 408;

Table 1. ^{13}C NMR data for methylated flavones from *Thymus vulgaris**

Carbon atom	Cirsilineol (**1**)	5, 6, 4'-Trihydroxy-7, 8, 3'-trimethoxyflavone (**2**)	5, 4'-Dihydroxy-6, 7, 8, 3'-tetramethoxyflavone (**3**)
2	163.87(*s*)	163.74(*s*)	163.44(*s*)
3	102.96(*d*)	102.65(*d*)	102.47(*d*)
4	182.13(*s*)	182.50(*s*)	182.01(*s*)
5	151.98(*s*)	152.46(*s*)	151.92(*s*)
6	131.67(*s*)	132.81(*s*)	135.30(*s*)
7	152.53(*s*)	141.78(*s*)	144.07(*s*)
8	91.55(*d*)	134.08(*s*)	132.08(*s*)
9	158.53(*s*)	143.00(*s*)	147.55(*s*)
10	105.02(*s*)	106.05(*s*)	105.69(*s*)
1'	121.34(*s*)	121.58(*s*)	120.86(*s*)
2'	110.18(*d*)	109.93(*d*)	109.51(*d*)
3'	147.97(*s*)	147.97(*s*)	147.97(*s*)
4'	150.83(*s*)	150.76(*s*)	150.52(*s*)
5'	115.70(*d*)	115.88(*d*)	115.40(*d*)
6'	120.37(*d*)	120.19(*d*)	119.88(*d*)
-OMe	56.00(*q*)	55.82(*q*)	55.27(*q*)
-OCMe	56.42(*q*)	60.91(*q*)	60.00(*q*)
-OMe	59.94(*q*)	61.76(*q*)	60.97(*q*)
-OMe			61.34(*q*)

*Spectra were recorded in DMSO-d_6 at 25 MHz. Chemical shifts were measured relative to the solvent signal and referred to TMS. Multiplicities in an off-resonance decoupling experiment are given in parentheses; *s* = singlet; *d* = doublet; *q* = quadruplet.

($NaOAc-H_3BO_3$) 274, 345. IR ν_{max}^{KBr} cm^{-1}: 3420, 2850, 1660, 1610, 1590, 1350, 1275, 835.

5, 6, 4′-Trihydroxy-7, 8, 3′-trimethoxyflavone (**2**): R_f values: A, 0.32; B, 0.49; C, 0.29; D, 0.54; E, 0.11; F, 0.72. ^1H NMR (100 MHz, DMSO-d_6): δ 3.88 (3H, *s*, –OMe), 3.92 (3H, *s*, –OMe), 3.94 (3H, *s*, –OMe), 6.92–7.08 (2H, *m*, H-3 and H-5′), 7.52–7.72 (2H, *m*, H-2′ and H-6′). EIMS 70 eV, *m/z* (rel. int.) 360 $[M]^+$ (68) for $C_{18}H_{16}O_8$, calc. 360.0845; found, 360.0815, 359 $[M-H]^+$ (5), 345 $[M-Me]^+$ (100), 197 $[A_1-Me]^+$ (15), 169 $[A_1-MeCO]^+$ (4), 151 $[B_2]^+$ (5) and 148 $[B_1]^+$ (< 2). UV λ_{max} nm: (MeOH) 250sh, 292, 343; (NaOMe) 259, 392; ($AlCl_3$) 241sh, 261, 306, 380; ($AlCl_3$–HCl) 240sh, 259sh, 305, 369; (NaOAC) 296, 310, 341, 392; ($NaOAc-H_3BO_3$) 296sh, 333. IR ν_{max}^{KBr} cm^{-1}: 3420, 2845, 1660, 1600, 1585, 1375, 1282, 840.

5, 4′-Dihydroxy-6, 7, 8, 3′-tetramethoxyflavone (**3**): R_f values: A, 0.44; B, 0.57;C, 0.42; D, 0.66; E, 0.43; F, 0.87. ^1H NMR (100 MHz, DMSO-d_6): δ 3.73 (3H, *s*, –OMe), 3.81 (3H, *s*, –OMe), 3.83 (3H, *s*, –OMe), 6.84–7.02 (2H, *m*, H-3 and H-5′), 7.52–7.66 (2H, *m*, H-2′ and H-6′). EIMS 70 eV, *m/z* (rel. int.) 374 $[M]^+$ (77) for $C_{19}H_{18}O_8$, calc. 374.1001; found, 371.0982, 373 $[M-H]^+$ (2), 359 $[M-Me]^+$ (100), 211 $[A_1-Me]^+$ (17), 183 $[A_1-MeCO]^+$ (10), 151 $[B_2]^+$ (4) and 148 $[B_1]^+$ (2). UV λ_{max} nm: (MeOH) 254, 281, 344; (NaOMe) 266, 409; ($AlCl_3$) 264sh, 287, 304sh, 375; $AlCl_3$–HCl: 261, 301sh, 365; NaOAc: 268, 414; $NaOAc-H_3BO_3$: 280, 346. IR ν_{max}^{KBr} cm^{-1} 3400, 2830, 1650, 1600, 1595, 1370, 1275, 840.

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