Phytochemistry, Vol. 24, No 8, pp 1869–1871, 1985
Printed in Great Britain

0031–9422/85 $3.00 + 0.00

5,6,4′-TRIHYDROXY-7,8-DIMETHOXYFLAVONE FROM *THYMUS MEMBRANACEUS*

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(*Received* 13 *November* 1984)

Key Word Index—*Thymus membranaceus*; Labiatae; 5,6,4′-trihydroxy-7,8-dimethoxyflavone; 5,8,4′-trihydroxy-6,7-dimethoxyflavone; 5,6,8,4′-tetrahydroxy-7-methoxyflavone; isomerization; demethylation.

Abstract—From the extracts of *Thymus membranaceus* subsp. *membranaceus*, the new naturally occurring 5,6,4′-trihydroxy-7,8-dimethoxyflavone (thymusin), has been isolated and identified. Its structure was elucidated by comparison with its isomeric flavone obtained on acid treatment (isothymusin); thymusin is demethylated at the 8-position during this treatment.

INTRODUCTION

As part of our program on the flavonoids of medicinal plants [1–3], we have now studied *Thymus membranaceus* Boiss. subsp. *membranaceus*, which is endemic to south-east Spain. The new naturally occurring flavone thymusin (**1**) (5,6,4′-trihydroxy-7,8-dimethoxyflavone), a compound previously obtained only by syntheses [4], has been isolated and characterized from extracts of this plant.

From *Thymus vulgaris* leaves, the new naturally occurring flavone thymonin (5,6,4′-trihydroxy-7,8,3′-trimethoxyflavone) was recently identified [5]. The differentiation between 5,8-dihydroxy-6,7-dimethoxyflavones and 5,6-dihydroxy-7,8-dimethoxyflavones is difficult by NMR techniques (lanthanide shift reagents and benzene induced shifts) [6, 7], and by the gossypetone reaction [8]. This last technique should be used with great caution as it may lead to erroneous conclusions, particularly when small quantities of compounds are available. We have already proposed a technique for the elucidation of these unusual structures by means of chromatographic and UV comparisons between the natural flavone and its isomeric form obtained by acidic treatment (Wessely–Moser rearrangement) [9, 10]. This technique has now been applied to the elucidation of the structure of this new flavone. The acidic treatment afforded, in addition to the Wessely–Moser isomers, a demethylation product.

RESULTS AND DISCUSSION

Compound **1** was isolated from the diethyl ether extract. Its UV spectrum in methanol suggested a flavone with a monosubstituted B ring (band I 334 nm), and a tetrasubstituted A ring with free hydroxyls at C-5 and C-6 (band II 297 nm) [10]. Addition of sodium methoxide to the methanolic solution showed a bathochromic shift of 43 nm with an increase in *A* indicative of a OH-4′ group [11]. The alkaline decomposition supported a 5,6- or 5,8-dihydroxyl system. The substitution of the OH-7 was shown by the absence of a substantial bathochromic shift in the sodium acetate band II (300 nm) relative to methanol (297 nm) [11] and by the maximum of band I in sodium acetate (384 nm) at a higher wavelength than in sodium methoxide (377 nm) [12]. The aluminium chloride–hydrochloric acid spectrum supported the presence of a free OH-5 group (band I bathochromic shift of 25 nm relative to methanol) and the possibility of another free OH-6 group [13, 14].

The chromatographic behaviour of **1** indicated it was a trihydroxydimethoxyflavone [15] and its permethylated derivative, obtained by diazomethane methylation, coincided with tangeretin (5,6,7,8,4′-pentamethoxyflavone), thus confirming the substitution pattern on the flavone nucleus. The electron impact mass spectrum (EIMS) of **1** exhibited a molecular ion peak at *m/z* 330 (69 %), in accord with a flavone containing three hydroxyls and two methoxyls. The retro-Diels–Alder (RDA) fragments confirmed the presence of one hydroxyl on ring B and two hydroxyls and two methoxyls on ring A (Table 1). The relative abundance of the peaks $[M]^+$ and $[M-Me]^+$, suggested the existence of a methoxyl at C-8 and the absence of a $[M-H_2O]^+$ peak confirmed this possibility [16, 17]. Thus, **1** is 5,6,4′-trihydroxy-7,8-dimethoxyflavone.

In order to confirm this structure we have obtained the isomeric compound **2**, by acidic treatment and Wessely–Moser rearrangement [9, 10]. Compound **2** had a higher R_f in 30 % acetic acid in cellulose (0.46) than **1** (0.29), supporting the presence of a free OH-8 group in **2** [9]. This was confirmed by the UV study of **2** in methanol, since this showed a very important band III at 306 nm (1.00) typical of 5,8-dihydroxyflavones, with a single substituent on ring B, and lower values for bands I and II relative to the methanol spectrum of **1** [9, 10]. The EIMS data confirmed that compound **2** is 5,8,4′-trihydroxy-6,7-dimethoxyflavone (isothymusin) [16, 17].

Acidic treatment yielded another flavone (**3**) in considerable yield, in addition of the two Wessely–Moser isomers. This compound was rather unstable and de-

composed slowly in solution, giving a reddish colour. Its R_f values suggested a highly hydroxylated flavone. This was in accordance with its great instability. The UV values in methanol suggested a single hydroxyl on ring B (band I at 335 nm) and a 5,8-dihydroxyl system (band III at 304 nm, 1.00). The aluminium chloride–hydrochloric acid spectrum (band I bathochromic shift of 31 nm relative to methanol), supported a free OH-5 group and an additional OH-6 group. The EIMS of **3** exhibited a molecular ion peak at m/z 316 (100%), in accord with a flavone containing four hydroxyls and one methoxyl. The RDA fragments showed the presence of one hydroxyl on ring B and three hydroxyls and one methoxyl on ring A. The low relative abundance of peak $[M-Me]^+$ (18%) and the importance of the $[M-H]^+$ (26%) and $[M-H_2O]^+$ (27%) peaks, suggested a Me-7 group. This was confirmed by acidic treatment of **3**, which yielded only one compound, according with the same substitution at C-6 and C-8. Compound **3** was produced by 8-demethylation of thymusin by acidic treatment. A 5-demethylation was reported previously, by means of an acidic treatment with acetic acid–hydrochloric acid [11].

EXPERIMENTAL

Plant material. Aerial parts of *Thymus membranaceus* Boiss. subsp. *membranaceus* were collected near Santomera (Murcia, Spain) and a voucher specimen is deposited in the herbarium of the Botany Department of the Facultad de Ciencias Biológicas at Murcia (No. 11.943).

Extraction. The air-dried powdered aerial parts (*ca* 0.5 kg) were exhaustively extracted at room temp. with EtOH–H_2O (7:3). This extract was concd under red. pres. until only the H_2O remained. The aq. concentrate was subjected to extraction with Et_2O.

Isolation of compound **1**. The Et_2O was removed and the dried extract was dissolved in C_6H_6–MeCOEt (19:1). This soln was chromatographed on polyamide SC-6 (Macherey–Nagel) first with C_6H_6–MeCOEt (19:1), the eluate being discarded and then with C_6H_6–MeCOEt (4:1). This second fraction was concd. Flavone **1** was isolated by prep. PC on Whatman No. 1 in 30% HOAc (R_f 0.48) and prep. TLC on silica gel with C_6H_6–HOAc (7:3) (R_f 0.32).

Chromatographic behaviour of **1**. Flavone **1** had the following R_f data [15]: on silica gel in C_6H_6–MeOH–HOAc (45:3:2) (R_f 0.25); in $CHCl_3$–C_6H_{14}–MeOH (40:40:3) (R_f 0.15); on polyamide DC-6 in H_2O–*n*-BuOH–MeCOEt–HOAc (7:1:1:1) (R_f 0.22); and in C_6H_6–petrol (80–100°)–MeCOEt–MeOH (60:26:7:7) (R_f 0.41).

Acid treatment Compound **1** was dissolved in MeOH and aq. 4 M HCl was added (1:1). This soln was heated in a stoppered tube, 4 hr, 100°. The MeOH was removed and the flavones extracted with EtOAc. Flavones **1** (40%), **2** (20%) and **3** (40%) were isolated from this EtOAc extract by prep. PC on Whatman No. 1 with 30% HOAc: R_f **1**, 0.48; R_f **2**, 0.58; R_f **3**, 0 36. Cellulose TLC 30% HOAc: R_f **1**, 0.29; R_f **2**, 0.46; R_f **3**, 0.18.

Thymusin (**1**) (*5,6,4'-trihydroxy-7,8-dimethoxyflavone*). UV λ_{max}^{MeOH} nm: 334 (1.00), 297 (0.96); + NaOMe, 377 (dec.), 330, 290 sh; + $AlCl_3$, 445 sh (0.15), 370 (1.00), 313 (0.85), 290i (0.52); + $AlCl_3$ + HCl, 440 sh (0.13), 359 (1.00), 311 (0.89), 287i (0.63); + NaOAc, 384, 355, 300; + NaOAc + H_3BO_3, 390 sh (0.25), 331 (0.78), 300 (1.00).

Isothymusin (**2**) (*5,8,4'-trihydroxy-6,7-dimethoxyflavone*). UV λ_{max}^{MeOH} nm: 370i (0.29), 330 sh (0.76), 396 (1.00), 288 sh (0.81); + NaOMe, 375 (dec.), 283; + $AlCl_3$, 450 sh (0.20), 357 (1.00), 323 (0.98), 289 (0.77); + $AlCl_3$ + HCl, 400 sh (0.27), 353 (0.83), 319

Table 1. EIMS of flavones **1–3** [*m/z* (relative abundance)]

Compound	$[M]^+$	$[M-H]^+$	$[M-Me]^+$	$[M-H_2O]^+$	$[M-CHO]^+$	$[M-H_2O-Me]^+$	$[M-MeCO]^+$	$[A_1-Me]^+$	$[A_1-MeCO]^+$	$[B_2]^+$	$[B_1+H]^+$	$[B_1]^+$
1	330	329	315	—	301	297	287	197	169	121	119	118
	(69)	(5)	(100)		(1)	(10)	(2)	(10)	(5)	(1)	(2)	(2)
2	330	329	315	312	301	297	287	197	169	121	119	118
	(100)	(9)	(97)	(7)	(4)	(23)	(5)	(21)	(10)	(4)	(5)	(6)
3	316	315	301	298	287	—	273	183	155	121	119	118
	(100)	(26)	(18)	(27)	(3)		(3)	(10)	(8)	(6)	(5)	(4)

The spectra were carried out by direct inlet samples (70 eV, 240° ion source temperatures and 280° probe temperature). Other fragments from **3** are: m/z 198 $[A_1]^+$ (3); m/z 197 $[A_1-H]^+$ (5).

(1.00), 286 (0.83); +NaOAc, 384, 320, 285 sh; +NaOAc +H_3BO_3, 330 sh (0.77), 305 (1.00), 285 (1.00).

8-Demethylthymusin (3) (*5,6,8,4'-tetrahydroxy-7-methoxyflavone*). UV λ_{max}^{MeOH} nm: 335 sh (0.69), 304 (1.00); +NaOMe, 469 (dec.), 378 (dec.), 330 (inc.), 247 sh; +$AlCl_3$, 468 (0.17), 400i (0.52), 372 (0.93), 320 (1.00); +$AlCl_3$ + HCl, 417 sh, (0.57), 366 (0.89), 311 (1.00); +NaOAc, 385 (dec.), 316 (inc.); +NaOAc + H_3BO_3, 430 sh (0.12), 304 (1.00).

Acknowledgements—We are grateful to the C.A.I.C.Y.T. of the Spanish Government for financial support of this work (grant No. 613/106) and to Dr. Antonio Arques for the mass spectral analysis and Dr. Francisco Alcaraz for Botanical surveillance.

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Phytochemistry, Vol 24, No 8, pp 1871–1872, 1985
Printed in Great Britain

0031–9422/85 $3.00 + 0.00

5,7-DIHYDROXY-3,8,3',4'-TETRAMETHOXYFLAVONE FROM *PARASTREPHIA QUADRANGULARIS*

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(*Received* 28 *November* 1984)

Key Word Index—*Parastrephia quadrangularis*; Compositae; Astereae; 5,7-dihydroxy-3,8,3',4'-tetramethoxyflavone.

Abstract—A new flavone has been isolated from *Parastrephia quadrangularis* and identified as 5,7-dihydroxy-3,8,3',4'-tetramethoxyflavone.

Five species of *Parastrephia* (Compositae, tribe Astereae) are distributed in northern Chile. Only one species *P. lepidophylla*, of Bolivian origin, has been chemically examined [1]. In this communication we report the isolation of a new flavone, characterized as 5,7-dihydroxy-3,8,3',4'-tetramethoxyflavone (**1**) as well as the identification of scopoletin, umbelliferone and *p*-coumaroyloxytremetone (**2**) from *P. quadrangularis* (Meyen) Cabrera.

Compound **1** had a molecular weight of 374 corresponding to a flavone with four methoxyl and two hydroxyl groups. The UV data indicated the presence of two hydroxyl groups at the 5 and 7 positions of the A ring and also suggested that C-8 but not C-6 was substituted by methoxy group. Thus, the shift of the short wave band in the sodium acetate spectrum compared to the methanol spectrum was 26 nm, which together with the shift of the long wave band in the $AlCl_3$–HCl spectrum (+74 nm) suggested a 5,7-dihydroxyflavone [2]. The ^1H NMR spectrum showed the typical signals for a 3',4'-substituted B ring (see Experimental): a signal for four methoxyl groups at δ3.85 and one-proton singlet at δ6.27. The