peak A 180 ($C_9H_8O_4$ calc. 180.0423, found 180.0422) confirmed the A ring configuration. This new natural compound is, thus, 5,7,3′-trihydroxy-6,8-di-*C*-methyl-4′,5′-dimethoxyflavanone and not, as described in ref. [8] and cited in ref. [11], as the B ring′isomer.

This is the first *C*-methylflavanone to be found in the Didiereaceae; the simultaneous presence of *O*- and *C*-methylation is typical in this family. Such *C*-methylated flavonoids are relatively rare in nature [12].

EXPERIMENTAL

Material: *Alluaudiopsis marnieriana* was collected in the South of Madagascar. 100 g of bark and spine powder was directly extracted with Et_2O. After evaporation of solvent, the dry residue was dissolved in MeOH and chromatographed on polyamide column (Macherey Nagel SC 6) with C_6H_6 progressively enriched in MeCOEt-MeOH (13). The ultimate purification of the fraction containing the new flavanone was assured by TLC on polyamide (MNDC 6) with C_6H_6-Petrol b.p. 100-140°-MeCOEt-MeOH (60-26-7-7). UV fluorescence: grey-violet; Rf (× 100): TLC, polyamide MNDC 11, $CHCl_3$-MeOH-MeCOEt-$AcCH_2Ac$: 60-10-5-1, 85.UV.λ_{max} nm: MeOH: 297, 348; + NaOAc: 340; + NaOAc + H_3BO_3: 299, 341; + $AlCl_3$:319, 410; + $AlCl_3$ + HCl: 317, 352 sh, 408; + NaOH: 340 stable. MS: 70 eV, *m/z* (%): 360 (85%), 207 (15%), 181 peak D (90%), 180 (100%, peak A (50%) + peak B (50%)), 167 (40%), 152 (30%). ^1H NMR 360 MHz Bruker (C_6D_6): δ6.69 (1H, *J* = 2 Hz); 6.67 (1H, *J* = 2 Hz); 5.34 (1H, *dd*, *J* = 12 Hz, *J* = 2 Hz); 3.80 (3H, *s*); 3.71 (3H, *s*); 3.04 (1H, *dd*, *J* = 15 Hz, *J* = 12 Hz); 2.75 (1H, *dd*, *J* = 15 Hz, *J* = 2 Hz)); 1.99 (3H, *s*); 1.97 (1H, *s*).

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6-HYDROXYFLAVONES FROM *THYMBRA SPICATA*

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Key Word Index—*Thymbra spicata*; Labiatae; 6-hydroxyflavones; 6-hydroxyluteolin 7,3′-dimethyl ether; 6-hydroxyluteolin 7,3′,4′-trimethyl ether.

Abstract—Four flavonoids, including two new compounds, were isolated from the leaf extract of *Thymbra spicata*. The new compounds were the 7,3′-dimethyl and 7,3′,4′-trimethyl ethers of 6-hydroxyluteolin. All the compounds were identified by spectral methods.

INTRODUCTION

This is the first chemical investigation of *Thymbra spicata*, a member of the Labiatae. From a leaf extract four flavonoids were identified: the known compounds luteolin and rhamnetin and two new 6-hydroxyflavones, **1** and **2**.

RESULTS AND DISCUSSION

One of the new flavones, **1** was previously reported as its 6-*O*-glucoside from *Citharexylum subserratum* (Verbenaceae) [1]. The following data established the structure of **1**. A molecular ion for the flavone at M^+ 330 indicated the presence of three hydroxyl and two methoxyl groups. The presence of hydroxyl groups at C-5, C-6 and C-4′ was supported by the somewhat unusual color reactions when the compound was viewed on paper under UV light with and without ammonia. A purple color under UV light indicated a 5-hydroxyl. The dark yellow color with ammonia supported, on the one hand, a 4′-hydroxyl, but the darkness of the spot also suggested the presence of a 6-hydroxyl. Compounds with a 6-hydroxyl group usually show little or no color change with

ammonia even when they contain a 4′-hydroxyl. The compound gave a brownish colour when sprayed with Naturstuffreagenz A. The presence of a 6-hydroxyl was also indicated by the aluminum chloride–hydrochloric acid UV spectrum which exhibited a band I shift of 26 nm relative to the methanol spectrum. The lack of a band III in the sodium methoxide spectrum along with the observation that band I in the sodium acetate spectrum appeared at 398 nm compared to band I in the sodium methoxide spectrum at 394 nm argued for a substituted 7-hydroxyl. The ^{1}H NMR spectrum of the 6,4′-diTMSi ether of **1** (i.e. 5-hydroxyl not derivatized) in $CDCl_3$ clearly established a 6-hydroxyluteolin dimethyl ether skeleton. The signals for the two methoxyl groups appeared at δ 3.92 and 3.94. Since the 7-hydroxyl is known from UV data to be substituted, one of the methyl ether groups could be assigned to the 7-hydroxyl. As the aluminum chloride UV spectrum indicated that the B-ring did not contain a 3′,4′-dihydroxyl group, the second methoxyl group must be at the 3′-position. All of the other UV, ^{1}H NMR and mass spectral data were in accord with the proposed structure. For example, other NMR signals appeared at δ 6.52 (1H, *s*, H-3), 6.58 (1H, *s*, H-8), 6.95 (1H, *d*, J = 9 Hz, H-5′), 7.32 (1H, *d*, J = 2.5 Hz, H-2′) and 7.42 (1H, *dd*, J = 9 and 2.5 Hz, H-6′). In the mass spectrum of **1** the $[M]^+$ at *m/z* 330 was the base peak; the presence of peaks at 312 (30%), $[M-H_2O]^+$; 183 (15%), $[A_1+1]^+$; 182 (12%), $[A_1]$; 149 (30%), $[B_2+1]^+$; and 148 (27%), $[B_2]^+$ confirmed that both the A and B rings contain one methoxyl group. That the $[M-15]^+$ peak was of low intensity and the $[M-18]^+$ peak was of greater intensity (30%), supported a free 6-hydroxyl group.

The second new compound, **2**, gave a molecular ion at M^+ 344 in accord with a flavone containing three methoxyl and two hydroxyl groups. The purple color exhibited by the compound when viewed on paper over UV light supported a free 5-hydroxyl group. In addition, since no color change was observed with ammonia or when sprayed with Naturstuffreagenz A, one methoxyl group could be assigned to the 4′-position. This conclusion was supported by a lower intensity band I in the sodium methoxide spectrum relative to band I in the methanol spectrum. The UV spectrum in aluminum chloride–hydrochloric acid showed a band I bathochromic shift of 26 nm, again, as for **1**, typical for a flavone with a free 6-hydroxyl. Since the ^{1}H NMR spectrum of the mono-TMSi ether of **2** (i.e. 5-hydroxyl not derivatized) established a 6-hydroxyluteolin trimethyl ether skeleton (methoxyl signals at δ 3.94, 3.98 and 4.0), the remaining two methoxyl groups must be at the only available positions, namely 7 and 3′. All of the other ^{1}H NMR, mass spectral and UV data supported the proposed structure as 6-hydroxyluteolin 7,3′,4′-trimethyl ether. Other signals were at δ 6.54 (1H, *s*, H-3), 6.58 (1H, *s*, H-8), 6.96 (1H, *d*, J = 9 Hz, H-5′), 7.34 (1H, *d*, J = 2.5 Hz, H-2′) and 7.5 (1H, *dd*, J = 9 and 2.5 Hz). The mass spectrum of **2** gave, in addition to the M^+ at 344 (100%), peaks at 326 (25%), $[M-18]^+$; 298 (55%), $[M-H_2O-CO]^+$; 182 (15%), $[A_1]^+$; and 162 (25%), $[B_2]^+$. These mass spectral data supported the presence of two methoxyl groups in the B ring and a third in the A ring. The known flavonoids rhamnetin and luteolin were identified by spectral data and by comparison with standard markers.

EXPERIMENTAL

Plant material was collected from Hatay (south-eastern Turkey) in late April 1980; voucher specimen ISTE 32979 was deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul.

Extraction, purification and identification of flavonoids from Thymbra spicata. Ground air-dried leaf material of *T. spicata* L. (0.5 kg) was extracted in a Soxhlet successively with C_6H_6, $CHCl_3$ and EtOH. Since the $CHCl_3$ and EtOH extracts did not contain flavonoids, only the C_6H_6 extract was worked-up. After evaporation of the C_6H_6 extract to a small vol. *in vacuo*, the concentrate was extracted with 60% aq. EtOH. The alcoholic layer was concd to a small vol. and extracted with Et_2O. The Et_2O extract was evaporated to dryness and the residue was fractioned on a Sephadex LH-20 column (2 × 30 cm) eluted with $CHCl_3$–EtOH (3:1). The flavonoids were obtained in the following order: rhamnetin (5 mg), luteolin (20 mg), 6-hydroxyluteolin 7,3′-dimethyl ether (20 mg), 6-hydroxyluteolin 7,3′,4′-trimethyl ether (40 mg). UV spectral data for **1** λ_{max}^{MeOH} nm with relative intensities based on the band I as 1 given in parenthesis: 343 (1), 282 (1), 272 (sh); NaOMe, 394 (1), 300 (sh), 275 (0.5); $AlCl_3$, 373 (1), 293 (1), 275 (0.7); $AlCl_3$–HCl, 369 (1), 293 (1), 275 (sh); NaOAc, 398 (1), 330 (1.2), 279 (1.4); NaOAc–H_3BO_3, 342 (1), 282 (1.1), 270 (sh).

UV spectrum of **2** λ_{max}^{MeOH} nm: 340 (1), 282 (0.9), 270 (sh); NaOMe, 316 (1), 288 (sh), 272 (sh); $AlCl_3$, 370 (1), 295(0.7), 260 (sh); $AlCl_3$–HCl, 366 (1), 294 (0.7), 255 (sh); NaOAc, 329 (1), 289 (sh), 270 (sh); NaOAc–H_3BO_3, 338 (1), 282 (0.9), 272 (sh).

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