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SANTOFLAVONE, A 5-DEOXYFLAVONOID FROM *ACHILLEA SANTOLINA*

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Key Word Index—*Achillea santolina*; Compositae; methoxylated flavones; flavonol methyl ethers; artemetin; 7-hydroxy-3,6,3′,4′-tetramethoxyflavone; santoflavone.

Abstract—Two methoxylated flavones isolated from the aerial parts of *Achillea santolina* were identified as 5-hydroxy-3,6,7,3′,4′-pentamethoxyflavone and the new compound, 7-hydroxy-3,6,3′,4′-tetramethoxyflavone. Their ^1H and ^{13}C assignments and structural characterization were achieved through analysis of the BB, DEPT, ^1H–^1H, COSY-45, HMQC and HMBC spectra.

INTRODUCTION

Achillea santolina L. is a herb found in Baluchistan and the North West Frontier Province of Pakistan [1]. It is widely used in folk medicine as a tonic, vermifugal and carminative. The plant also relieves stomach pain [2]. Pharmacological screening of the plant showed antibacterial and antifungal activities. In the present study two flavones were isolated, a quercetagetin derivative **1** [3, 4] and a new *O*-methyl derivative of 5-deoxyflavonol rhynchosin [5] named santoflavone (**2**).

RESULTS AND DISCUSSION

The aerial parts of the *A. santolina* were extracted with methanol. The methanolic extract was evaporated, the residue loaded onto a silica gel column and eluted with a gradient of petroleum ether in ethyl acetate. The fraction obtained with petroleum ether–ethyl acetate (1:1) was then subjected to flash chromatography and two flavones, **1** and **2**, were isolated.

Compound **1** had a UV spectrum consistent with that of flavonoids [6]. The two peaks at λ_{max} 253 and 275 nm in band II, and the peak at λ_{max} 342 nm indicated that **1** is a C-3 substituted flavonol with oxygenation at C-5, C-6, C-7 and C-4′ [6, 7]. The intensity of the NaOMe spectrum did not decrease with time signifying the absence of *ortho* di-OH groups in both rings A and B. An absorption of band I at 332 nm further showed that **1** was a 3-methoxyl rather than a 3-hydroxyl flavone. The UV spectrum obtained in acidic $AlCl_3$ gave a bathochromic shift of + 60 nm in band I, indicating a free C-5 hydroxyl with oxygenation at C-6. Methoxyl groups both at C-3 and C-6 were also signified in this spectrum by the peak at 284 nm in band II and a shoulder at 402 nm in band I [6]. The IR spectrum of **1** showed peaks at 3510, 2800, 1720 and 1650 cm^{-1} which corresponded to hydroxylic and ketonic groups. The high resolution mass spectrum of **1** corresponded to the molecular formula $C_{20}H_{20}O_8$, with M_r 388, which is indicative of a five methoxyl and one hydroxyl substitution. A fragment ion at *m/z* 345 $[M - 43]^+$ confirmed methoxyl substitution at C-3 [8, 9]. A peak at *m/z* 197 (4%) due to a $[A_1 + H]^+$ fragment showed the presence of two methoxyl groups and one hydroxyl group in ring A, whereas the $[B_2]^+$ peak at *m/z* 165 (39%) indicated two methoxyls in ring B. The peak at *m/z* 345 (13%) representing the $[M - COMe]^+$ fragment showed an intensity of only 13%. Thus the loss of this fragment must be due to the methoxyl at C-3 and not C-6, as in the latter case the expected intensity of the peak would be much stronger [9]. This was also supported by the fact that a strong peak was observed at *m/z* 373 (64%), due to a $[M - 15]^+$ fragment. This loss can be either from the C-6 methoxyl or the C-8 methoxyl [8]. In the absence of the latter, the loss of the methyl radical must be from the C-6 methoxyl. The ^1H NMR ($CDCl_3$) exhibited a double doublet at δ7.72 (J = 8.5, 1.97 Hz) showing an *ortho* and *meta* coupling, a doublet at δ7.67 (J = 1.97 Hz) and another doublet at δ6.97 (J = 8.5 Hz) showing *meta* and *ortho* coupling, respectively. A singlet at δ6.49 was due to the proton at C-8. A singlet for C-5 hydroxyl proton was observed at δ12.6 which had been earlier reported in the ^1H NMR spectrum recorded in DMSO-d_6 only [10]. The spectroscopic data indicated that **1** was identical to artemetin, 5-hydroxy-3,6,7,3′,4′-pentamethoxyflavone [4]. The ^{13}C NMR data are listed in Table 1. The chemical shifts were assigned by comparison with similar compounds [10–13] and also with artemetin [14].

The UV spectrum of **2** showed two characteristic flavonoid bands in the range 300–380 and 240–280 nm [6]. The two bands at 339 and 275 nm were consistent with C-3, C-6, C-3′ and C-4′ oxygenated flavones. There was no shift of band II on addition of NaOAc indicating

Table 1. ^{13}C NMR chemical shifts of **1** ($CDCl_3$) and **2** ($CDCl_3$)

C	1	2
2	155.8	153.1
3	138.9	132.7
4	178.9	182.0
5	152.3	104.4
6	132.4	158.8
7	158.8	164.0
8	90.4	90.6
9	152.8	153.4
10	105.7	106.1
1′	123.8	123.8
2′	110.0	108.9
3′	148.9	149.4
4′	151.5	152.4
5′	111.5	111.2
6′	122.2	120.1
3-OMe	60.8	60.8
6-OMe	60.1	56.3
7-OMe	56.3	—
3′-OMe	56.0	56.1
4′-OMe	56.2	56.2

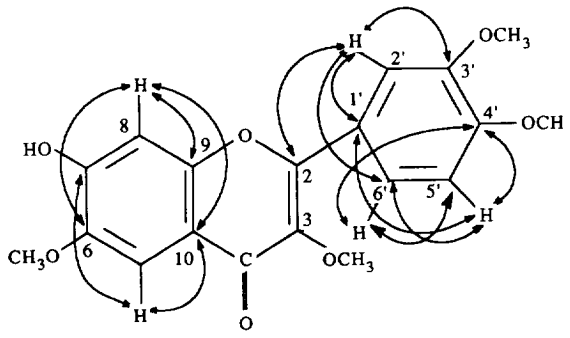

Fig. 1. Long-range correlations detected by the HMBC spectrum of santoflavone (**2**).

either the absence of a 7-hydroxyl group or that the 7-hydroxyl group had electron-donating substitution in its vicinity, which had reduced its acidity [6]. The $AlCl_3$ mixed spectrum of the compound showed a very small bathochromic shift of 8 nm which indicated that there were no *ortho* dihydroxyl groups in any of the rings. The methanol spectrum was totally restored after addition of dilute HCl, which confirmed the absence of C-5 and/or C-3 hydroxyl groups. The IR spectrum of 2 showed peaks at 3530 and 1720 cm^{-1} which are characteristic of hydroxylic and ketonic groups, respectively. The high resolution mass spectrum of **2** corresponded to the molecular formula $C_{19}H_{18}O_7$ and a M_r of 358. A fragment ion at *m/z* 315 $[M - 43]^+$ confirmed methoxyl substitution at C-3 [4, 8, 9]. The ^1H NMR spectrum exhibited a double doublet at δ7.50 (J = 8.46, 1.9 Hz) showing an *ortho* and *meta* coupling, a doublet at δ7.31 (J = 1.9 Hz) and a doublet at δ6.95 (J = 8.5 Hz) showed a *meta* and an *ortho* coupling, respectively, consistent with a B ring having C-3′, C-4′ substitution. Two singlets at δ6.53 and 6.58 were due to protons at C-8 and C-5, respectively, which confirmed substitution at C-6 and C-7. However, the singlet at δ6.58 assigned to H-5 in **2** was upfield as compared to δ7.38 (1H, *s*, H-5) in rhynchosin [5], but this was supported by the HMQC spectral data of our new compound **2**. One bond ^1H–^{13}C connectivities were observed between δ_H6.58 with δ_C104.4 (C-5), and also δ_H6.53 with δ_C90.6 (C-8).

The ^1H NMR further confirmed the presence of four methoxyl groups with signals at δ3.97,* 3.96,* 3.95* and 3.91 (3,3′,4′ and 6-OMe) (*assignments interchangeable). The spectral data of **2** indicated that it has the structure 7-hydroxy-3,6,3′,4′-tetramethoxyflavone. The HMBC spectrum showed the correlation of H-5 (δ_H6.58) with C-10 (δ_C106.1), C-7 (δ_C164); H-8 (δ_H6.53) with C-10 (δ_C106.1), C-9 (δ_C153.4), C-6 (δ_C158); H-2′ (δ_H7.31) showed cross peaks with C-2 (δ_C153.1), C-1′ (δ_C123.8), C-3′ (δ_C149.4), C-6′ (δ_C120.1); H-5′ (δ_H6.95) with C-4′ (δ_C152.4), C-6′ (δ_C120.1), C-1′ (δ_C123.8) and similarly H-6′ (δ_H7.50) showed correlation with C-4′ (δ_C 152.4) and C-5′ (δ_C111.2). The HMBC correlations are shown in Fig. 1.

EXPERIMENTAL

Plant material: Fresh plant material of *Achillea santolina* L. was collected in April, 1992 from Baluchistan, University campus area. A sample of the plant was authenticated by Dr. R. B. Tareen of the same University and a voucher specimen No. 212 is deposited in the herbarium of the Botany Department, Baluchistan University. For CC, silica gel 60 of mesh 230–270, and for flash chromatography, silica gel 60 mesh 230–400 (Art No. 9385) were used. Mass spectrometry: MAT-112 S and JEOL HX-110. NMR spectra: Bruker AC-300.

General. Plant material (2 kg, aerial parts) was extracted with MeOH. Repeated chromatographic sepn of the methanol-soluble extract (200 g) was done on silica gel column and flash chromatographs. The eluents used were; petrol → petrol–EtOAc. The flavonoid spots were visualized under UV light (365 nm) and by spraying with 1% $AlCl_3$ in EtOH. The purity of the compounds was checked by TLC using the system petrol–EtOAc (3:2) for **2**. For **1** the solvent system petrol–EtOAc (1:1) was used.

Artemetin (**1**). Yellow crystals, mp 147°; $[\alpha]_D^{25°}$ − 13.3° (MeOH; *c* 0.15); UV λ_{max}^{MeOH} 253, 275, 342; + NaOMe 253sh, 291, 332, 393sh (intensity does not decrease with time); MeOH + $AlCl_3$, 263, 283sh, 297sh, 372; + $AlCl_3$/HCl, 261, 284, 359, 402sh; MeOH + NaOAc 254, 273, 343; + H_3BO_3 255, 273, 344 EIMS: M^+ (%) 388 (100), 373 (64), 345 (13), 343 (16), 315 (8), 197 (4), 165 (39), 69 (62), 57 (64). IR ($CHCl_3$) cm^{-1}: 3530, 2850, 1720, 1650, 1590, 1140, 1000; ^1H NMR ($CDCl_3$): δ7.72 (1H, *dd*, J = 8.5, 2.12 Hz, C-6′), 7.67 (1H, *d*, J = 2.08 Hz, C-2′), 6.97 (1H, *d*, J = 8.58 Hz, C-5′), 6.49 (1H, *s*, C-8), 3.85 (3H, *s*, C-3, OMe), 3.91 (3H, *s*, C-6, OMe), 3.95* (3H, *s*, C-7, OMe), 3.96* (3H, *s*, C-4′, OMe), 3.96* (3H, *s*, C-3′, OMe) (* assignments interchangeable).

Santoflavone (**2**). Brown crystals, mp 150°; $[\alpha]_D^{25}$ + 16.4 (MeOH; *c* 12.2); UV λ_{max}^{MeOH} 255sh, 275, 339; + NaOMe 253sh, 278, 333, 407sh; MeOH + $AlCl_3$, 257, 275, 348; $AlCl_3$/HCl, 256, 275, 338, 408sh, MeOH + NaOAc 253sh, 276, 339; + H_3BO_3 253sh, 275, 339, 407sh; IR ($CHCl_3$) cm^{-1}: 3500, 2810, 1720, 1650, 1590, 1120, 1005; MS: M^+ (%), 358 (100), 343 (81), 315 (25), 167 (23), 165 (25), 83 (76), 55 (86); ^1H NMR ($CDCl_3$): δ7.50 (1H, *dd*, J = 8.46, 1.9 Hz, C-6′), 6.95 (1H, *d*, J = 8.51 Hz, C-5′), 7.31 (1H, *d*, J = 1.9 Hz, C-2′), 6.53 (1H, *s*, C-8), 6.58 (1H, *s*, C-5), 3.91 (3H, *s*, C-3, OMe), 3.95* (3H, *s*, C-6, OMe), 3.96* (3H, *s*, C-4′, OMe), 3.97* (3H, *s*, C-3′, OMe) (*assignments interchangeable).

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