ISOLATION AND CHARACTERISATION OF FLAVONOIDS FROM *ARTEMISIA CAMPESTRIS L.SUBSP. GLUTINOSA* PLANT

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Résumé. Deux nouvelles structures moléculaires de type flavonoïde ont été isolées d'une plante m édicinale, Artémisia campestris, le 3, 5, 3'-trihydroxy -7,4'- diméthoxy-6-[3-méthylbut-2-ényl] flavone (1) et le 5-hydroxy-7,2',4',5'- tetraméthoxy flavone (2). Les structures ont été caractérisées par les techniques spectroscopiques.

Abstract. Two new flavonoids were isolated from the medicinal plant Artemisia campestris, 3,5,3'-trihydroxy -7,4'-dimethoxy-6-[3-methylbut-2-enyl]flavone (1) and 5-hydroxy-7,2',4',5'-tetramethoxy flavone (2). The structures were characterized by spectroscopic methods.

Introduction.

Artemisia campestris L.SUBSP glutinosa gay (compositae) (ref.1,2,3) is a tubuliflorus plant. It is and arido-activated perennial chamephyte widespread in Djeffara region (Tunisia) (ref.4). Preliminary examination of crude extracts of this species which is uninvestigated from a chemical point of view revealed neutralization of the scorpion venon effect, antimicrobial activity and inhibition of the germination process of some herbal species (ref.4). We now wish to report the structure of two novel flavonoïds isolated from this plant.

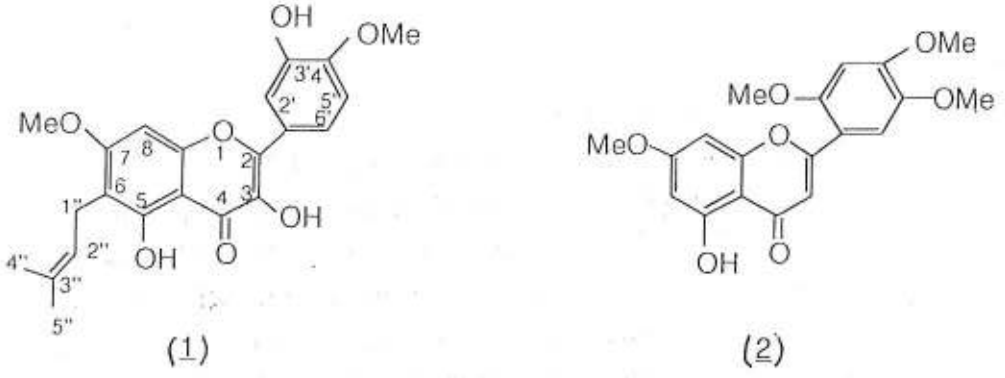

Figure 1. Structure of Flavones (1) and (2).

Results and Discussion:

The petroleum ether extract from the aerial parts of Artemisia campestris was chromatographed over Silica gel to yield flavone (1).

Through UV spectral analysis a bathochromic shift of 60 nm with $AlCl_3$ showed the presence of 3-OH groups. Absence of bathochromic shift with NaOMe showed substitution at the 7 and 4' position. Its ^{1}H-NMR spectrum exhibited two singlets at δ 3.90 and 3.85 indicating two MeO groups. The presence of a prenyl residue was evidenced from the signals at δ 5.15 (vinyl proton), 3.42 (allylic protons) and 1.68, 1.76 (vinyl methyls). The singlet at δ 6.58 was assigned to H-8. The signal at 7.70 is due to H-2'. The NOESY spectrum of (1) indicated that the methoxyl group (δ 3.85) enhanced the aromatic proton signal (δ 3.90). The eims of (1) gave the molecular ion peak at m/z 398 and fragments at m/z 179 and 151, which indicated the presence of a prenyl group in ring A . The structure as 3,5,3'-trihydroxy 7,4'-dimethoxy-6-[3-methyl but-2-enyl] flavone was in agreement with the ^{13}C-NMR spectral data. Compound (1) was shown to be identical in all respects with a sample of the dehydro-derivative of isotirumalin (ref.5).

5-hydroxy-7,2',4',5'-tetramethoxy flavone (2), was obtained as faint yellow solid from the recrystallization ($CH€l_3$) . The spectral data revealed that (2) belongs to the flavone group of compounds. It possesses four methoxyl groups as evidenced from its ^{1}H-and ^{13}C-NMR spectra (3.87 ppm, s, 3H ; 3.93, s, 6H; 3.97, s, 3H ; 55.8, 56.1, 56.3, 56.8 ppm).

In addition 2 was indicated a hydroxyl group at position 5 as apparent from the low-fields ^{1}H-NMR chemical shift (δ 12.92) of this proton due to strong hydrogen bonding with the carbonyl group.

The absence of ortho-coupling expected for the 5-hydroxy - 7,2',4',5'- tetramethoxy flavone confirmed the 2',4',5' isomer. The NOESY spectrum of (2). indicated that the methoxyl group (δ 3.97) enhanced the two proton signals (δ 6.35 H-6 and 6.45 H-8), the aromatic proton (δ 7.40 H-3') enhanced the methoxyl signals (δ 3.93 and 3.87) and the aromatic proton (δ 6.58 H-6') enhanced the methoxyl signal (δ 3.93).

Experimental

General Experimental Procedures.

- NMR experiments were performed on a varian XL-400 (400 MHz-for ^{1}H NMR and 100,6 MHz for carbon) using TMS as an internal standard. All the samples for NMR analyses were dissolved in $CDCl_3$. UV spectra were recorded on a Perkin-Elmer Lambda 17 spectrophotometer, IR on 5 DXC FT-IR spectrophotometer using KBr discs. Mass spectra were determined on a Hitachi M80 instrument, mps on an MRK air-bath type melting point apparatus and are uncorrected. Silica gel (Merck type 60,70-230 mesh) was

used for analytical tlc. Detection of components was made by use of a UV lamp and heating after spraying with 10% aqueous H_2SO_4.

Plant Material.

- Artemisia campestris characterized by a pleasant odor was collected in July 1994 at Djeffara region, south of Tunisia.

The identification was performed by Ing. Mohamed Neffati from the Arid Region Institute. Voucher specimens are deposited in the Department of Agronomy, ARI.

Extraction and isolation.

- Air - dried, powdered aerial parts of A. Campestris 1500g were macerated with petroleum ether (b.p 30-60°) for 2 days. Evaporation of petroleum ether at reduced pressure supplied 22g dark green extract. This extract was fractioned on a Si gel column (Si gel 200 - 300 mesh) and eluated with petroleum ether - Et_2O (100:1, 50:1, 25:1, 5:1). Flavonoïds-containing fraction (2.2g) was collected when the Rf [Si gel G, petroleum ether - Et OAc (5 : 1)] of the eluate is 0.70 - 0.76. Compounds 1 and 2 were isolated by preparative paper chromatography on what man n° 3MM paper. in toluene - $CHCl_3$ - Me OH (5.5 : 3.0 : 1.5). The band at Rf 0.82 yielded pale yellow needles recrystallized with MeOH, (1) (200mg). The band at Rf 0.51 gave white crystals recrystallized by using $CHCl_3$, 2 (172mg).

3,5,3'-Trihydroxy-7,4'-dimethoxy- 6-[3-methyl but-2-enyl] flavone (1). - Mp 190.5 Found C 66.21, H 5.42 ; $C_{22}H_{22}O_7$ requires C 66.32, H 5.57%. UV λ max (MeOH) nm (log ε) 262 (4.22), 272 sh (4.18), 310 sh (3.91), 382 (4.20), + $AlCl_3$ 271, 310 sh, 363, 442, +$AlCl_3$/HCl 271, 310 sh, 363, 442, +NaOMe 272, 350 sh, 435, +NaOAc 262, 410 ; IR (KBr)cm^{-1}3520, 3240, 1650, 1620, 1598, 1550, 1420, 1355, 1260; eims m/z 398[M^+] (100), 383 [M-CH_3]$^+$ (84), 369 (6), 368 (3), 367 (3), 343 (11), 330 (45), 315 (6), 179 (6), 151 (14); ^{1}H NMR (400 MHz, $CDCl_3$) δ12.50 (1H, s, 5-OH),7.76 (1H, dd, 8.0 and 2.5Hz, H-6'), 7.70 (1H, d, 2.5 Hz, H-2'), 7.10 (1H, d, 8Hz, H-5'), 6.58 (1H, s, H-8), 5.15 (1H, m, H-2"), 3.90 (3H, s, 4'-OMe), 3.85 (3H, s, 7-OMe), 3.42 (2H, d, 8.0 Hz, H-1"), 1.76 and 1.68 (6H, 2s, Me-4" and Me-5")-; ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 176.0 (C-4), 164.9 (C-7), 160.4 (C-5), 156.0 (C-9), 149.5 (C-4'), 146.7 (C-2), 146.2(C-3'), 136.2(C-3), 130.5 (C-3"), 123.2 (C-1'), 122.4 (C-2"), 119.7 (C-6'), 114.7 (C-2'), 114.5 (C-5'), 110.2 (C-6), 103.5 (C-10), 94.8 (C-8), 56.2 (2xOMe), 25.5 (Me), 21.2 (C-1"), 17.7 (Me).

5-Hydroxy - 7,2',4',5'- tetramethoxy flavone 2.-White solid ; Mp 182.5; Found C 63.56, H 4.92; $C_{19}H_{18}O_7$ requires C 63.68, H 5.06% .

UV ($CHCl_3$) λ max (log ε) 260 (4.35), 291 (4.02), 360 (4.31) nm ; (kBr) max 2920s, 2850 m, 1652vs, 1614vs, 1583 vs, 1563s, 1505 vs, 1468s, 1446 s, 1391m, 1365s,

1271 vs, 1210vs, 1162 vs, 1119m, 1027 s 984w, 946w, 851m, 807m cm^{-1}; ^1H NMR δ 3.87 (3H, s, 2'-OMe), 3.93 (6H, s, 2XMeO), 3.97 (3H, s, 7-OMe), 6.35 (1H, d, J= 2.2 Hz H-6), 6.45 (1H, d, J = 2.2 Hz, H-8), 6.58 (1H, s, H-6'), 7.04 (1H, s, H-3), 7.40 (1H, s, H-3'),12.92 (1H, s, OH); ^{13}C NMR δ 55.8 (MeO), 56.1 (MeO), 56.3 (MeO), 56.8 (MeO), 92.4 (C-8), 97.1 (C-6'), 97.8 (C-6), 105.5 (C-10) 109.7 (C-3), 111.4 (C-1'), 111.9 (C-3'), 109.7(C-3), 111.4(C-1'), 111.9(C-3'), 143.1 (C-4'), 152.8 (C-2') 154.0 (C-5'), 157.7 (C-5), 161.0 (C-2), 162.1 (C-9), 165.3 (C-7), 182.8 (C-4); eims m/z 358 $[M]^+$(100), 343 $[M-CH_3]^+$ (11), 315 $[M-COCH3-]^+$ (11),167 (37).

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