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FLAVONOL GLYCOSIDES FROM *Centaurea furfuracea.* ANTIPLASMODIAL AND CYTOTOXIC ACTIVITIES

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Many species of the genus *Centaurea* (Asteraceae) have been used in folk medcine since ancient times. These plants are generally rich in flavonoids and sesquiterpene lactones, which are the main constituents responsible for biological activity [1–6]. In a previous study, we described 10 flavonoids from *Centaurea furfuracea* [6, 7].

C. furfuracea Coss. et kral. was collected at the beginning of April 1992 in the region of Biskra and was identified by Dr. M. Kaabech from the University of Setif Algeria on the basis of Quezel and Santa [8]. A voucher specimen has been deposited in the herbarium of the Museum National d′Histoire Naturelle, Paris, France.

Powdered dried aerial parts from *C. furfuracea* were extracted with MeOH. The MeOH extract was concentrated. The concentrate was taken up with $CHCl_3$ and *n*-BuOH. The latter, by preparative PC on Whatman 3MM paper using 15% AcOH and BAW (*n*-BuOH–AcOH–H_2O 4:1:5), upper phase as solvents, gave compounds **1** and **2**.

Purification of each compounds for spectral analysis was carried out using Sephadex LH-20; column eluted with MeOH; prior to UV, MS, and ^{1}H NMR spectral analysis, color reaction procedure; hydrolysis of the glycosides (0.1 N HCl, 2 h) (Mabry et al.) [9, 10].

Ultraviolet and visible absorption spectra were determined with a Perkin–Elmer Lamda 17 UV/VIS spectrophotometer. Absorption maxima were obtained in methanol. Spectra shifts were recorded after adding a standard solution of sodium hydroxide, aluminium chloride, hydrochloric acid, and crystals of sodium acetate.

Compounds **1** and **2** by treatment with 2 N HCl (2 h, 100°C) gave glucuronic acid and the aglycone identified as Me-3 kaempferol (isokaempferide).

The structures were established on the basis of NMR spectroscopic experiments and by comparison of the NMR data. Compound **2** was reported previously from another species of *Centaurea* [11], and from *C. furfuracea* for the first time. The glycoside **1** is new for the genus *Centaurea*.

The UV spectra of **1** were typical of flavone or 3-*O* substituted flavonol. After adding 2 N NaOH, it showed no band shift between 320 and 335 nm, whereas band II was not shifted by adding NaOAc. This was in agreement with substitution of the 7-hydroxyl group [12]. The shift of band I in the sodium hydroxide spectrum of compound **1** indicated a bathochromic shift with increased intensity and supported the presence of a free 4′-hydroxyl group. The negative Electrospray MS of compound **1** exhibited a quasi-molecular ion $[M-H]^-$ at *m/z* 489, suggestive of the empirical formula $C_{23}H_{22}O_{12}$. The ^{1}H NMR spectrum of compound **1** obtained in CD_3OD and the AA′BB′ system consists of two proton doublets (J = 8.4 Hz) at δ 7.94 and 6.90 typical of a *para*-substituted B ring of flavonoid. Two further coupled doublets (J = 2 Hz) at δ 6.80 and 6.55 indicated the presence of the *meta*-related H-6 and H-8 ring A protons. The presence of two methoxyl groups was shown by two 3H singlets at δ 3.85 and 3.92. The presence of a sugar moiety was revealed by the anomeric proton resonance (δ 5.30, d, J = 8 Hz). Therefore, **1** was identified as isokaempferide 7-*O*-methylglucuronide. The other isolated compound gave the isokaempferide 7-*O*-glucuronide (**2**).

Isokaempferide 7-*O*-Methylglucuronide (1). $C_{23}H_{22}O_{12}$, UV/Vis (MeOH): λ_{max} 272, 333 nm; NaOH: 252, 274, 385; $AlCl_3$: 279, 357; $AlCl_3$/HCl: 280, 350; + NaOAc 270, 390 nm. ^{1}H NMR (300 MHz, CD_3OD, J/Hz): 7.94 (2H, d, J = 8.4, H-2′, H-6′), 6.90 (2H, d, J = 8.4, H-3′, H-5′), 6.80 (1H, d, J = 2, H-8), 6.55 (1H, d, J = 2, H-6), 3.92 (3H, s, COOMe), 3.85 (3H, s, OMe-3), 5.30 (1H, d, J = 8, anomeric proton of glucuronic methylester H-1″), 4.23 (1H, d, J = 10, H-5″ CH-COOMe), 3.30–3.70

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(3H, m, H-2″, H-3″, H-4″). ES-MS negative ion mode *m/z*: 489 $[M-H]^-$, 299 [M-190(Megluc) -H]$^-$; ES-MS positive ion mode *m/z*: 491 $[M+H]^+$, 301 [M-190(Megluc) +H]$^+$.

Isokaempferide 7-*O*-Glucuronide (2). $C_{22}H_{20}O_{12}$, UV/Vis (MeOH): λ_{max} 267, 345; NaOH: 252, 270, 395; $AlCl_3$: 274, 356, 399; $AlCl_3$/HCl: 273, 351, 398 nm. ^{1}H NMR (300 MHz, CD_3OD, J/Hz): 7.95 (2H, d, J = 8.4, H-2′, H-6′), 6.96 (2H, d, J = 8.4, H-3′, H-5′), 6.85 (1H, d, J = 2, H-8), 6.56 (1H, d, J = 2, H-6), 3.81 (3H, s, OMe-3), 5.50 (1H, d, J = 8, anomeric proton of glucuronic acid H-1″) 4.25 (1H, d, J = 10, H-5″ CH-COOH), 3.25–3.75 (3H, m, H-2″, H-3″, H-4″, H) ES-MS positive ion mode *m/z*: 477 $[M+H]^+$, 301 [M-176(gluc) +H]$^+$.

Antiplasmodial, Cytotoxic Activities. Antiplasmodial and cytotoxic activities were evaluated according to established protocols [13, 14].

Tested on Plasmodium falciparum, the chloroform extract exhibited antiparasitic activity (IC_{50} 7.94 μg/mL). On KB cells, it showed cytotoxic activity with a growth inhibition of 90% at 10 μg/mL and 26% at 1 μg/mL.

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