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A NEW FLAVONOID GLYCOSIDE FROM *DICTAMNUS ALBUS*

CHR. SOULELES

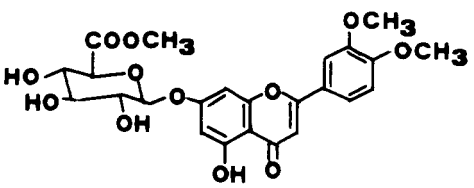
Laboratory of Pharmacognosy, Department of Pharmacy, University of Thessaloniki, 54006 Thessaloniki, Greece

ABSTRACT.—A new flavonoid glycoside, luteolin 3′,4′-dimethyl ether-7-*O*-β-D-methylglucuronide [**1**] was isolated from the leaves of *Dictamnus albus*. In addition, two known flavonoid methyl ethers, luteolin 7,3′-dimethyl ether and luteolin 3′-methyl ether, were isolated. Structures were elucidated by chemical and spectroscopic methods.

The present work is part of a phytochemical investigation on *Dictamnus albus* L. (Rutaceae). This plant grows in Greece and is known by the common name of "racobotano" (1). It has been used in Greek folk medicine as an antispasmodic, tonic, stimulant, and antihelmintic. This work concerns the isolation and characterization of a new flavonoid glycoside and two known methylated flavones.

The three flavonoids were isolated from the EtOAc fraction and were identified as luteolin 3′,4′-dimethyl ether-7-*O*-β-D-methylglucuronide [**1**], luteolin 7,3′-dimethyl ether, and luteolin 3′-methyl ether. The absence of a shift of band II (240, 269 nm) in the uv spectrum with NaOAc confirmed the 7-*O*-substitutions. Spectra recorded in the presence of $AlCl_3$ + HCl and NaOAc + H_3BO_3 indicated the absence of free 3′,4′-*ortho*-dihydroxyl systems. The presence of a double peak for band II (IIa 269, IIb 240) in each indicated that the C-3′ and C-4′ positions are substituted (2).

The ^{1}H-nmr spectrum of **1** showed an aromatic zone identical to that of luteolin 3′,4′-dimethyl ether, as well as three 3-proton singlets at δ = 3.85, 3.75, and 3.65 ppm (2 Ar-OMe and -CO_2Me) and two 1-proton doublets at δ = 4.20 (J = 9.0 Hz) and 5.32 ppm (J = 7.0 Hz) characteristic of O-CH-CO_2Me and an anomeric proton of a sugar, respectively. These data and the appearance of the molecular ion at *m/z* 504 were consistent with a luteolin 3′,4′-dimethyl-methylglucoronide. Acid hydrolysis of **1** gave an aglycone identified as luteolin 3′,4′-dimethyl ether by comparison of chromatographic and spectroscopic (uv, ^{1}H-nmr) data with data found in Mabry *et al.* (3), and a sugar, identified as the methyl ester of glucoronic acid by comparison on pc with an authentic sample. The absence of a bathochromic shift of band II with NaOAc in the uv absorption and the presence of one phenolic hydroxyl at C-5 in the ^{1}H-nmr spectrum (signal at δ = 12.92 ppm) suggested that the methylglucuronide must be attached to the hydroxyl at C-7. Because the coupling constant of the anomeric proton was characteristic of a *trans*-diaxal coupling, the structure of luteolin 3′,4′-dimethyl ether-7-*O*-β-D-methylglucuronide was assigned to compound **1**.



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The structures of luteolin 7,3′-dimethyl ether and luteolin 3′-methyl ether were determined by comparing chemical and spectroscopic (uv, ^{1}H-nmr) data with data found in Sakakibara *et al.* (4) and Souleles and Laskaris (5).

EXPERIMENTAL

PLANT MATERIAL.—Leaves of *D. albus* were collected in Chalkidiki, Greece in May 1987. The plant was authenticated by the Botanical Museum of the University of Thessaloniki, where a voucher specimen of the plant has been deposited. The samples were dried in a cool dark place and coarsely powdered.

GENERAL EXPERIMENTAL PROCEDURES.—Spectra were recorded with the following instruments: uv-vis, Perkin-Elmer 554; ^{1}H-nmr, Brücker AW 80 MHz; ms, Hitachi-Perkin-Elmer-61 (70 ev); pc, on Whatman no. 3MM. Solvents: *n*-BuOH–HOAc–H_2O (3:1:1) (TBA); HOAc 15%. Cc: Polyamide-SC-6-(MN). Spot reagents: NH_3 vapor and Naturstoffreagenz-A 1% in MeOH. Cyanidin test: MeOH sol + Mg + HCl.

ISOLATION AND IDENTIFICATION OF COMPOUNDS.—Dried plant material (1200 g) was succesively extracted with petroleum ether, $CHCl_3$, and MeOH in a Soxhlet extractor. The solvents were evaporated in vacuo leaving 5.1 g, 12.2 g, and 20.1 g of residues, respectively. The residue of the MeOH extract was partioned between H_2O and three organic solvents: $CHCl_3$, EtOAc, and *n*-BuOH. Extracts were evaporated to dryness. The EtOAc extract (6.5 g) was chromatographed over a polyamide column eluted with a mixture of MeOH and H_2O. Fractions were further fractionated and purified by pc on Whatman no. 3MM in solvents TBA and 15% HOAc. This yielded in a pure state the following compounds: luteolin 3′,4′-dimethyl ether-7-*O*-β-D-methylglucuronide [**1**] (48 mg), luteolin 7,3′-dimethyl ether (35 mg), and luteolin 3′-methyl ether (32 mg).

LUTEOLIN 3′,4′-DIMETHYL ETHER-7-*O*-β-D-METHYLGLUCURONIDE [**1**].—Yellow compound, Cyanidin test yellow. Color on paper under uv (366 nm), purple; uv/NH_3, yellow; uv/Naturstoffreagent-A, yellow-green. Uv λ max (MeOH) 240, 269, 290 sh, 341, (NaOMe) 275, 315, 370, ($AlCl_3$) 260, 275, 300, 358, 385, ($AlCl_3$/HCl) 258, 279, 295, 348, 381 sh, (NaOAc) 240, 269, 341, (NaOAc/H_3BO_3) 245, 341; ^{1}H-nmr (DMSO-d_6) δ 12.92 (s, 1H, OH-5), 7.33 (d, J = 2 Hz, 1H, H-2′), 7.38 (dd, J = 2 Hz, 8 Hz, 1H, H-6′), 6.88 (d, J = 8 Hz, H-5′), 6.93 (s, 1H, H-3), 6.85 (d, J = 2.4 Hz, 1H, H-8), 6.45 (d, J = 2.4 Hz, 1H, H-6), 5.55 (m, 3H, OH-3), 5.32 (d, J = 7.0 Hz, 1H, H-1″), 4.20 (d, J = 9.0 Hz, 1H, H-5″), 3.85, 3.75 (s, 2ArOMe) and 3.65 (s, 3H, CO_2Me); ms (FI-CAI) *m/z* $[M]^+$ 504, $[M-31]^+$ 473, $[M-32]^+$ 472, $[M-64]^+$ 442, [aglycone]$^+$ 314.

HYDROLYSIS OF **1**.—Compound **1** was hydrolyzed with 5% HCl for 2 h under reflux to yield luteolin 3′,4′-dimethyl ether (co-pc, uv, ^{1}H-nmr) and the methyl ester of D-glucuronic acid. The sugar was identified by pc against standard markers in TBA, using saturated aqueous solution of aniline reagent.

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