Vitexin, esculetin and isomollupentin were identified by UV, MS and standard sample comparisons.

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FLAVONE 5-*O*-GLUCOSIDES FROM *DAPHNE SERICEA*

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Key Word Index—*Daphne sericea*; Thymelaeaceae; flavones; flavone 7- and 5-*O*-glucosides; isovitexin; coumarins; a steroidal glycoside; antitumor activity.

Abstract—The aerial parts of *Daphne sericea* yielded two new flavonoids, luteolin 7-methyl ether 5-β-D-glucoside and luteolin 7,3′-dimethyl ether 5-β-D-glucoside, as well as luteolin 7-methyl ether, isovitexin, apigenin and its 7-β-D-glucoside.

INTRODUCTION

Since *Daphne mezereum* (family Thymelaeceae) was previously found to contain the antileukemic diterpenoid mezerein [1–3], an extract of the aerial parts of another *Daphne* sp., namely *D. sericea* Vahl, was tested for antitumor activity and its chemical constituents investigated. Except for two new flavone 5-O-glucosides, the compounds isolated from *D. sericea* are typical of other *Daphne* sp. For example, in other studies the coumarins umbelliferone, daphnerotin, daphnetin and 7-hydroxycoumarin 8-β-D-glucoside were isolated from *D. mezereum* [4] as was the toxic diterpene daphnetoxin [5]. *D. odora* afforded the flavones luteolin and apigenin [6] and the coumarins daphnin and daphnetin [7], two compounds which were also found in both *D. pontica* [8] and *D. acuminata* [9]. The latter species also contained daphnetin 8-β-D-glucoside [10]. *D. cannabina* yielded daphnerotin and β-sitosterol [11], while the diterpenoid yuanhuacine has been isolated from *D. genkwa* [12].

RESULTS

The aqueous layer which remained after partitioning the concentrate from the ethanol extract of *Daphne sericea* with organic solvents was tested for its antitumor activity and found to be active against the 3PS *in vivo* test system. However, the compounds so far isolated from this fraction, including the coumarins daphnerotin and daphnin, sitosteryl 3-β-D-glucoside and several flavonoids, apparently do

not account for this activity. No diterpenoids were detected in the active concentrate. The main component isolated from this aqueous concentrate was luteolin 7-methyl ether with lesser amounts of its 5-glucoside and the 5-glucoside of luteolin 7,3'-dimethyl ether.

Another species in the Thymelaeaceae *Ovidia pillopillo* was previously found to contain a similar 5-*O*-glucoside, namely luteolin 7-methyl ether 5-xylosylglucoside [13].

The known compounds were identified by UV, MS (except daphnin), IR (daphnin, daphnerotin, sitosteryl 3-glucoside), acetylation (sitosteryl 3-glucoside) and standard sample comparisons. The structural assignments of the new compounds are discussed separately.

Luteolin 7-methyl ether 5-β-D-glucoside

This new glycoside appeared blue on paper over UV light (366 nm) turning blue-green with NH_3 vapor. With NA reagent the compound also gave a blue color in UV light. Both acid (0.1 N TFA) and β-glucosidase hydrolyses gave an aglycone (plus glucose) which appeared purple in UV light turning yellow with NH_3 vapor and orange with NA reagent. The aglycone was identified as luteolin 7-methyl ether by UV, MS and TLC comparison with a standard. Confirmation that the natural product is a 5-*O*-glucoside was provided by its UV and the ^1H NMR (90 MHz, CCl_4) of its TMSi ether. In addition to typical signals for a luteolin skeleton [H-3, δ6.4; H-6, 6.45 (*d*, $J = 2.5$); H-8, 6.55 (*d*, $J = 2.5$); H-2' and -6', 7.2 (*m*); H-5, 6.86 (*d*, $J = 9$)], the ^1H NMR spectrum exhibited signals typical for a monomethyl ether monoglucoside. The methoxyl signal, which appeared at δ3.9, gave a C_6H_6-induced upfield shift of Δ 0.55 ppm typical for 7-methoxyl groups. The spectrum also exhibited signals for seven glucosyl protons including H-1'' at δ5.15. The UV spectra further confirmed the structural assignment. Band I in the $AlCl_3$ spectrum appeared at 387 nm and shifted to 345 nm with $AlCl_3$/HCl, a value essentially identical to Band I in the spectrum of the compound in methanol alone; these results indicated the presence of a 3',4'-dihydroxyl system along with a blocked 5-hydroxyl. Furthermore, absence of a Band III (no low intensity peak or shoulder between 305–345 nm) in the NaOMe spectrum and the lack of a shift in Band II in the NaOAc spectrum indicated a 7-*O*-substituent. Together, these spectral and hydrolytic findings established that the new glycoside is luteolin 7-methyl ether 5-β-D-glucoside.

Luteolin 7,3'-dimethyl ether 5-β-D-glucoside

The second new flavone glycoside gave the same color reactions as the first; however, the aglycone appeared yellow with NA over UV light. Since acidic (0.1 TFA) and β-glucosidase hydrolyses yielded glucose and luteolin 7,3'-dimethyl ether (UV, MS), the compound must be luteolin 7,3'-dimethyl ether 5-β-D-glucoside. Its UV and the ^1H NMR spectrum of its TMSi ether confirmed this structure; the latter spectrum exhibited signals similar to those obtained for the first compound except for two methoxyl resonances (at δ3.9 and 3.95) rather than one.

*Relative absorptivities are given for each λ_{max} relative to the longest wavelength band as 1.

EXPERIMENTAL

Plant material. Aerial parts of *Daphne sericea* Vahl (Thymelaeaceae) were collected from southern Turkey, Antakya (Harbiye district) in March 1976. A voucher specimen (ISTE 34486) is deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul.

Extraction and isolation. The dried, powdered plant material (1 kg) was extracted with EtOH in a Soxhlet. After concentrating to a mostly aq. soln. the soln was successively partitioned with *n*-hexane, C_6H_6 and $CHCl_3$. Although all the extracts were concentrated and tested against the LE and 3PS (P388 lymphocytic leukemia) test systems only the aq. layer remaining after partitioning showed any antitumor activity and only against the 3PS system [*T/C* 182 for 100 mg/kg; 152 for 75 mg/kg; 138/45 mg/kg]. The aq. concentrate (10 g) was fractionated on a Si gel column (5 × 60 cm). Elution of the column was initiated with C_6H_6 and the polarity was gradually increased by the addition of $CHCl_3$, then EtOH up to 100%. The compounds were obtained in the following order: daphnin (4 mg), daphnerotin (5 mg), sitosteryl 3-glucoside (10 mg) and a mixture of flavonoids (5 mg), luteolin 7-methyl ether (120 mg), apigenin (5 mg), column (4 × 50 cm). Elution of the column was initiated with $CHCl_3$–EtOH (2 : 1) and the polarity of the eluate was gradually increased by reducing the amount of $CHCl_3$. The flavonoids were obtained in the following order: isovitexin (5 mg), luteolin 7-methyl ether (120 mg)[3], apigenin (5 mg), luteolin 7-methyl ether 5-glucoside (25 mg), luteolin 7,3'-dimethyl ether 5-glucoside (20 mg) and apigenin 7-glucoside (10 mg).

Luteolin 7-methyl ether 5-β-D-glucoside. UV λ_{max}^{MeOH} nm: 344 (1),* 304 (sh), 260 (sh), 247 (sh), 240 (1.1); + NaOMe 404 (1), 290 (0.1), 260 (sh), 238 (sh); + $AlCl_3$ 378 (1), 300 (sh), 257 (sh); + $AlCl_3$/HCl 408 (sh), 345 (1), 300 (sh), 263 (sh), 250 (sh); + NaOAc 420 (sh), 365 (1), 300 (0.2), 256 (0.2); + NaOAc/H_3BO_3 365 (1), 305 (0.1), 252 (1.1). TLC (cellulose plates): R_f 0.23 (15% HOAc); 0.32 (40% HOAc); and 0.40 (*t*-BuOH–HOAc–H_2O) (3 : 1 : 1). MS of underivatized comp. M^+, 300; $(A_1 + 1)$, 167; B_1, 134; B_2, 137.

Luteolin 7,3'-dimethyl ether 5-β-D-glucoside. UV λ_{max}^{MeOH} nm: 340 (1), 300 (sh), 262 (sh), 240 (0.8); + NaOMe 402 (1), 290 (0.1), 253 (0.5), 240 (sh); + $AlCl_3$ 342 (1), 300 (sh), 262 (sh), 242 (0.9); $AlCl_3$/HCl 342 (1), 300 (sh), 262 (sh), 240 (1.1); + NaOAc 405 (sh), 350 (sh), 300 (0.1), 262 (sh), 250 (sh); + NaOAc/H_3BO_3 343 (1), 300 (sh), 262 (sh), 240 (sh). TLC (cellulose plates): R_f 0.17 (15% HOAc); 0.25 (40% HOAc); and 0.53 (TBA). MS of underivatized comp. M^+, 314; $(A_1 + 1)$, 167; B_1, 148.

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A METHYLATED FLAVONE FROM *ARTEMISIA MESATLANTICA*

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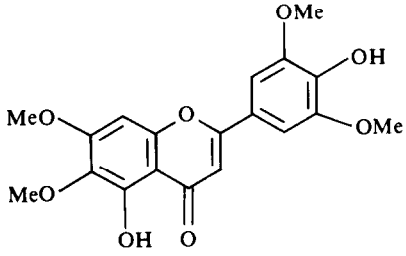
Key Word Index—*Artemisia mesatlantica*; Compositae; Anthemideae; new flavone; 5,4'-dihydroxy-6,7,3',5'-tetramethoxyflavone.

Abstract—From the aerial parts of *Artemisia mesatlantica*, a new highly methoxylated flavone was isolated. Its structure was determined by spectroscopic methods as 5,4'-dihydroxy-6,7,3',5'-tetramethoxyflavone.

INTRODUCTION

As part of our investigations on *Artemisia mesatlantica* Maire (N. Bouzid, C. Moulis and I. Fouraste, unpublished results), we report here the isolation of seven flavonoids. One of these, 5,4'-dihydroxy-6,7,3',5'-tetramethoxyflavone or 7-methyl-6-methoxytricin is a new natural compound.



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RESULTS AND DISCUSSION

The Et_2O extract of air-dried aerial parts afforded the new compound **1** after chromatographic separations. Its molecular formula, $C_{19}H_{18}O_8$ (MS 374,100) was in accord with a flavone containing two hydroxyl and four methoxyl groups, The ^1H NMR spectrum displayed three singlets at δ3.76 (3H), δ3.92 (6H) and δ3.98(3H) indicating the four methoxyl groups. Substitution at C-7 was demonstrated by the failure of Band II to show a bathochromic shift in NaOAc relative to MeOH alone [1]. The $AlCl_3$ shift in Band I of 24 nm indicated the presence of a free 5-hydroxyl function, and implied a 5-OH,6-OR grouping [2]; as the λ_{max} of Band I was lower than 280 nm in MeOH and appeared as a single peak with $AlCl_3$, the compound was substituted at C_6 by a methoxyl group and at C-3 by a proton (B. Voirin, unpublished results).

The A-ring structure was compatible with ^1H NMR which exhibited two singlets respectively at δ7.04 and 7.10 for H_3 and H_8 and with MS, ion fragment at *m/z* 181 ($C_9H_9O_4$) according to Audier [3]. The location of the B-ring hydroxyl and two methoxyl groups was established by UV data (the NaOMe reagent