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COUMARINS AND FLAVONOIDS FROM *DAPHNE GNIDIOIDES*[1]

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Daphne species (Thymelaeaceae) have shown a variety of pharmacological actions. Mezerein obtained from *Daphne mezereum* (1-4) has shown antileukemic activity; odorocin is a nematocidal compound from *Daphne odora* (5); and daphnetin 8-glucoside from *Daphne acuminata* (6) possesses cardiotoxic activity. The roots of *Daphne genkwa* (7) have been used against schistosomiasis, and flavonoids from *Daphne papyracea* (8) have shown sedative and hypotensive effects. Yuanhuafin obtained from the flowers of *D. genkwa* (9-11) is used as an abortifacient in China.

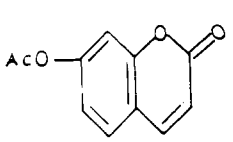
In order to find new active compounds, we initiated a pharmacological and chemical study with *Daphne* species grown in Turkey (12). *Daphne gnidioides* Jaub et Spack is a native plant collected from the Aegean coastal region. Although its crude extract showed a slight inhibition of the growth of cell cultures of L-cells (13), none of the compounds isolated in this study was found to be active[2]. We established a slight antibacterial activity for the isolated flavonoids using standard strains of *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, and Klebsiella pneumoniae* (14-16), while the coumarins did not inhibit bacterial growth. In this study, we report the isolation of five known coumarins: daphnin, daphnetin, daphnetin 8-glucoside, daphnoretin, and esculin; a diterpenoid daphnetoxin; six flavonoids: apigenin 7-glucoside, luteolin 7-glucoside, luteolin 4′-glucoside, isovitexin, vicenin 2, and quercetin 3-glucoside; and three terpenic compounds: β-amyrin, β-sitosterol, and sitosteryl 3-β-glucoside. The identities of the known compounds were established by spectral data (uv for flavonoids, ir for terpenoids, ^{1}H nmr and ms for coumarins), and tlc comparison with authentic samples. We have also isolated two new compounds, acetylumbelliferone (**1**) and demethyldaphnoretin (**2**).

The ms of **1** exhibited a molecular ion peak at *m/z* 204 indicating a molecular formula of $C_{11}H_8O_4$. The ir spectrum showed the presence of an acetyl group (1725, 1220 cm^{-1}) and another carbonyl absorbance at 1745 cm^{-1}. No hydroxyl peak was observed. The ^{1}H-nmr spectrum clearly showed the structure of **1**, with signals at δ 7.67 (1H, d, J=9 Hz, H-4), 7.47 (1H, d, J=9 Hz, H-5), 7.10 (1H, d, J=2 Hz, H-8), 7.03 (1H, dd, J=9 Hz and 2 Hz, H-6), 6.20 (1H, d, J=9 Hz, H-3), and 2.32 (3H, s, $OCOCH_3$).

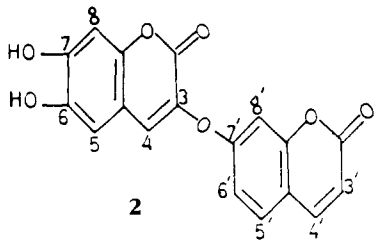
The mass spectrum of **2** exhibited a molecular ion peak at *m/z* 338 indicating a molecular formula of $C_{18}H_{10}O_7$. The ir spectrum showed hydroxyl bands at 3400 cm^{-1} and a carbonyl at 1740 cm^{-1}. The ^{1}H-nmr spectrum suggested

[1]A part of this study was presented in the poster session of 33rd annual Congress of the Society for Medicinal Plant Research (Regensburg, W. Germany.

[2]Cell culture tests were conducted by Prof. Dr. Atilla Özalpan, Faculty of Sciences, University of Istanbul.



1



2

the structure as illustrated for compound **2**: δ 8.04 (1H, d, J=9 Hz, H-4′), 7.82 (1H, s, H-4), 7.70 (1H, d, J=9 Hz, H-5′), 7.38 (1H, s, H-5), 7.18 (1H, d, J=2.5 Hz, H-8′), 7.1 (1H, dd, J=9 Hz and 2.5 Hz, H-6′), 6.85 (1H, s, H-8), and 6.36 (1H, d, J=9 Hz, H-3′).

EXPERIMENTAL

PLANT MATERIAL.—*D. gnidioides* was collected from the Aegean coast (Fethiye) of Turkey in June 1984, and was identified by one of us (E. Tuzlacı). A voucher specimen is deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul (ISTE 51424).

INSTRUMENTS.—Spectra were recorded with the following instruments: uv, Varian Techtron 635; ir, Perkin-Elmer 577; ^{1}H nmr, Nicolet FT-NT 300 MHz; and ms, Varian MAT 311.

ISOLATION AND IDENTIFICATION OF THE COMPOUNDS.—Air-dried and powdered leaves and stems of the plant (1 kg) were extracted with C_6H_6, $CHCl_3$, and EtOH. Upon evaporation under vacuum, 60 g, 22 g, and 200 g residues were obtained, respectively. The C_6H_6 extract, when separated on a Si gel column (5×70 cm), yielded β-amyrin (11 g) and β-sitosterol (1 g) by eluting with C_6H_6 and a gradient of $CHCl_3$ (to 100%). From Si gel columns of the $CHCl_3$ (10 g) and EtOH (10 g) extracts, coumarins were obtained. Elution was initiated with $CHCl_3$ and EtOH being added gradually up to 100%. Flavonoids and sitosteryl 3-β-glucoside were isolated from a Polyclar column of the EtOH extract, eluting with $CHCl_3$-EtOH (2:1) and the polarity being increased by decreasing the amount of $CHCl_3$. The flavonoids obtained from Polyclar column were cleaned over Sephadex LH-20 using EtOH for elution.

ACETYLUMBELLIFERONE (**1**).—Mp 135-137°; yield 1 g; uv λ max (EtOH) 325 nm (log ε 4.15), 263 (log ε 3.7); ir ν max (KBr) 3050, 1745, 1725, 1690, 1625, 1410, 1380, 1220, 1130, 910, 860 cm^{-1}; ^{1}H nmr ($CDCl_3$) given in the text; ms (probe) 70 eV, *m/z* (rel. int.) 204 $(M)^+$ (43.3), 162 $(M-CH_2CO)^+$ (93.3), 134 $(M-CH_2CO-CO)^+$ (93.4), 105 $(M-CH_2CO-CO-CHO)^+$ (48.7).

DEMETHYLDAPHNORETIN (**2**).—Amorphous; yield 10 mg; uv λ max (EtOH) 323 nm (log ε 4.2), 268 (log ε 3.7), 210 (log ε 4.8); ir ν max (KBr) 3400, 3050, 1740, 1680, 1620, 1550, 1445, 1400, 1280, 1130, 1070, 1030, 830 cm^{-1}. ^{1}H nmr (DMSO-d_6) given in the text; ms (probe) 70 eV, *m/z* (rel. int.) 338 $(M)^+$ (42), 320 $(M-H_2O)^+$ (10), 310 $(M-CO)^+$ (30), 177 $(C_9H_5O_4)^+$ (50), 161 $(C_9H_5O_3)^+$ (24).

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