Phytochemistry, Vol. 26, No. 11, pp. 3051–3053, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00

A CYTOTOXIC TETRALONE DERIVATIVE FROM *PARARISTOLOCHIA FLOS-AVIS*

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(*Received* 23 *February* 1987)

Key Word Index—*Pararistolochia flos-avis*; Aristolochiaceae; flossonol; (−)-phillygenin.

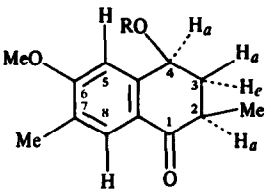
Abstract—A new tetralone derivative and a new lignan, namely flossonol and (−)-phillygenin, respectively, were isolated from *Pararistolochia flos-avis*. Their structures were elucidated on the basis of NMR, MS, UV, and IR spectral data. (−)-Sesamin and (−)-eudesmin were also isolated from this plant. The new tetralone was cytotoxic to PS cells in culture.

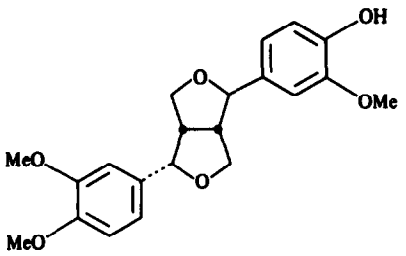
INTRODUCTION

In the course of a continuing search for tumour inhibitors from higher plant sources, a previous investigation [1] established the occurrence in the root and stem of *Pararistolochia flos-avis* Hutch & Dalz of four aristolactams, **FI**, **FII**, **I** and **AII**. In this report a new tetralone derivative and a new lignan, named flossonol (**1**) and (−)-phillygenin (**2**) respectively, and the known compounds, (−)-sesamin (**3**) and (−)-eudesmin (**4**) have been isolated from the active neutral fraction of the same plant. The new tetralone (**1**) was cytotoxic against PS cells in culture [2].

RESULTS AND DISCUSSION

Flossonol (**1**) has a molecular formula $C_{13}H_{16}O_3$ based on high resolution mass spectra data. Its IR spectrum indicated the presence of hydroxyl (3490 cm^{-1}) and carbonyl (1670 cm^{-1}) absorption bands. Its UV spectrum had absorptions at 225 (log ε 3.88), 274.5 (3.89) and 300 nm (sh 3.68) suggesting an acetophenone chromophore [3]. The 470 MHz ^{1}H NMR spectrum of **1** showed an aromatic methyl group at δ2.21 (3H, *s*), a methoxyl group at δ3.91 (3H, *s*) and two uncoupled aromatic protons at δ6.77 (1H, *s*) and 7.83 (1H, *s*), respectively. The

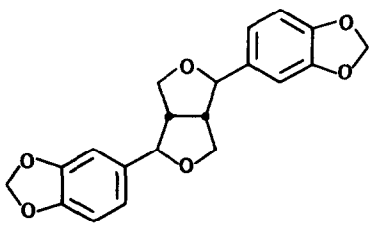




1 $\xrightarrow[Py]{Ac_2O}$ **1a**

(R = H) (R = Ac)

2



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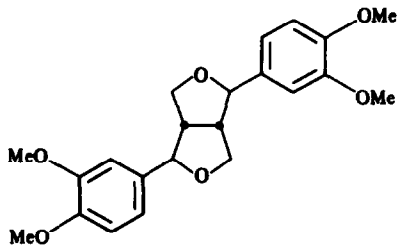

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NMR spectrum also indicated the presence of an aliphatic methyl group at δ1.46 (3H, *d*, J = 5) and a hydroxyl group at δ3.93 (1H, *s*, disappeared on addition of D_2O). Spin-decoupling experiments confirmed that the methylene protons at δ1.78 (1H, *ddd*, J = 12,12,12) and at 2.48 (1H, *ddd*, J = 12,5,5) were coupled with the C-2 methine proton on the carbon bearing methyl group at δ3.17 (1H, *ddq*, J = 12,5,5) and the C-4 methine proton on the carbon bearing hydroxyl group at δ4.33 (1H, *dd*, J = 12,5). Analysis of the position of the hydroxyl group eliminated the possible 2-hydroxy-4-methyl keto isomer (no H-bond). The placement of the hydroxyl group at C-4 was confirmed by irradiation of the proton on the carbon bearing hydroxyl group (δ4.33) which produced a 7.8% NOE on H-5 (δ6.77). The above data showed that flossonol had structure **1** in which the methyl and methoxyl substituents on the aromatic ring were either as shown in structure **1** or were reversed. The UV absorption band at 274 nm suggests the 6-methoxy-7-methyl tetralone structure over the 7-methoxy-6-methyl isomer, since calculated absorption bands for the two isomers are at 277 and 266 nm respectively [3]. In addition, the 20% NOE on the C-8 proton (δ7.83) and the 25% NOE on the C-5 proton (δ6.77) were observed upon irradiation of the methyl (δ2.21) and methoxyl (δ3.91) protons, respectively. The relative configuration of **1** was assigned based on the coupling constants of Ha-4 (δ4.33), Ha-2 (δ3.17), Ha-3 (δ1.78) and He-3 (δ2.48). Acetylation of **1** gave the monoacetate **1a**. Comparison of the chemical shifts of Ha-3 and He-3 in **1** and **1a** showed an upfield shift of 0.12 ppm for He-3, a downfield shift of 0.21 ppm for Ha-3, and no shift on the Me-2 group, consistent with a structure in which the 2-methyl and 4-hydroxy (or acetoxy) groups are 1,3-*cis* and diequatorial. Thus the structure of flossonol (**1**) is 3,4-dihydro-4-hydroxy-6-methoxy-2,7-dimethylnaphthalen-1(2H)-one. Flossonol (**1**) showed cytotoxicity at $3.4 \times 10^{\circ}$ μg/ml against PS cells in culture [2].

The other new compound, (−)-phillygenin (**2**), had a molecular formula $C_{21}H_{24}O_6$ based on HRMS data. Its IR and NMR spectra were identical with those reported in the literature [4, 5] for the lignan (+)-phillygenin. These compounds were shown to be enantiomers by comparison of their optical rotations.

Compounds **3** and **4** were identified as (−)-sesamin and (−)-eudesmin by comparison of their spectral data and physical properties with literature value [6–9].

EXPERIMENTAL

Plant material. Root and stem of *Pararistolochia flos-avis* were obtained from Ghana in March 1977, and authenticated by the Medicinal Plant Resources Laboratory Plant Genetics and Germplasm Institute, Building 265, Agricultural Research Center, East Beltsville, Maryland.

General experimental procedures. Mps: uncorr; UV: EtOH; IR: KBr; High resolution (470 MHz) ^{1}H NMR: $CDCl_3$ (δ in ppm, J in Hz) using TMS as int. stand.; 200 MHz ^{1}H NMR and 50.3 MHz ^{13}C NMR: fully decoupled and gated decoupled; low and high resolution MS: 70 eV. Silica gel (230–400 mesh size) was used for flash CC. Fractions were combined on the basis of their TLC patterns detected by UV light (250 and 360 nm).

Extraction and isolation. A previous investigation [1] established the fractionation of the antileukaemic active neutral fraction, which yielded 7 fractions (A–G) by flash chromatography on a silica gel column. Fraction A (5.48 g) was treated with a solvent mixture of hexane and Et_2O to yield a solid which was recrystallized from Et_2O to give compound **3** (2.12 g). Fraction B (7.5 g) was subjected to chromatography on a silica gel flash column eluted with hexane and increasing concentrations of EtOAc (10–50%) in hexane, 6 fractions (M–R) were collected on the basis of TLC. Fraction N was crystallized with a solvent mixture of hexane and Et_2O and afforded compound **3** (0.5 g). Fraction O was separated on the chromatotron using a silica gel plate eluted with 20% and 30% EtOAc in hexane to give a solid which was recrystallized from a mixture of Et_2O and hexane to yield compound **1** (104.1 mg). Fraction Q was recrystallized from EtOAc and hexane to give compound **4** (77.1 mg). Fraction R was subjected to prep. TLC (silica gel, EtOAc–hexane, 1 : 1) to give a solid which was recrystallized from EtOAc–hexane to give compound **2** (10 mg).

Flossonol (**1**). Mp. 93–95°, IR ν_{max}^{KBr} cm^{-1} 3490, 2930, 1670, 1610, 1565, 1490, 1250; HRMS m/z 220.1104 (calcd $C_{13}H_{16}O_3$ 220.1095); EIMS m/z (rel. int.): 220 [M]$^+$ (38), 202 (12), 189 (5), 176 (100), 159 (10), 148 (36), 133 (37), 115 (24); ^{1}H NMR: see Results.

Acetylation of flossonol (**1**). Compound **1** (2.8 mg) was acetylated with Ac_2O (0.4 ml) and C_5H_5N (0.4 ml) at room temp. for 10 hr and yielded the monoacetate **1a**, 470 MHz ^{1}H NMR ($CDCl_3$): δ1.46 (3H, *d*, J = 5), 1.99 (1H, *ddd*, J = 12,12,12), 2.20 (3H, *s*), 2.21 (3H, *s*), 2.36 (1H, *ddd*, J = 12,5,5), 3.23 (1H, *ddq*, J = 12,5,5), 3.91 (3H, *s*), 5.52 (1H, *dd*, J = 12,5), 6.75 (1H, *s*), 7.83 (1H, *s*).

(−)-*Phillygenin* (**2**). Mp 128–130° (EtOAc–hexane); $[\alpha]_D^{20}$ −114° ($CHCl_3$, *c* 0.1); HRMS m/z: 372.1582 (calcd for $C_{21}H_{24}O_6$, 372.1566); EIMS m/z (rel. int.): 372 [M]$^+$ (19), 355 (42), 337 (5), 325 (8), 249 (100), 235 (82), 219 (44), 205 (48), 189 (37), 175 (27), 151 (59), 137 (72); IR ν_{max}^{KBr} cm^{-1} 3450, 1600, 1585, 1510, 1455, 1260; ^{1}H NMR ($CDCl_3$) δ2.89 (1H, *m*), 3.32 (2H, *m*), 3.80–3.86 (3H, *m*), 3.88 (3H, *s*), 3.90 (6H, *s*), 4.42 (1H, *d*, J = 7), 4.87 (1H, *d*, J = 5), 5.57 (1H, *s*), 6.85–6.93 (6H, *m*).

(−)-*Sesamin* (**3**). Mp 119–121° (EtOAc–hexane, mp 123°, EtOH) [6], $[\alpha]_D^{20}$ −60° ($CHCl_3$; *c* 0.75); IR ν_{max}^{KBr} cm^{-1} 2820, 1610, 1480, 1435, 1250, 1080, 1020, 780; HMRS m/z 354.1104 (calcd $C_{20}H_{18}O_6$ 354.1098). EIMS m/z (rel. int.): 354 [M]$^+$ (11), 337 (13), 325 (8), 307 (6), 233 (100), 215 (3), 203 (78), 187 (8), 173 (10), 135 (71); 200 MHz ^{1}H NMR ($CDCl_3$): δ3.05 (2H, *m*), 3.86 (2H, *dd*, J = 3.8, 8.3), 4.23 (2H, *dd*, J = 6.8, 8.3), 4.71 (2H, *d*, J = 3.8), 5.59 (4H, *s*), 6.78–6.84 (6H, *d*, J = 11); 50 MHz ^{13}C NMR ($CDCl_3$) δ54.2, 71.6, 85.7, 101, 106, 108, 119.3, 134.9, 147, 147.9.

(−)-*Eudesmin* (**4**). Mp 105–107° (EtOAc–hexane) (mp 108.5–109.5° (EtOH–EtOAc) [8]); $[\alpha]_D^{22}$ −63.5° (Me_2CO; *c* 1.0); IR ν_{max}^{KBr} cm^{-1} 2940, 2910, 2820, 1590, 1580, 1500, 1455, 1435, 1250; HRMS m/z 386.1718 (calcd $C_{22}H_{26}O_6$ 386.1722); EIMS m/z (rel. int.): 386 [M]$^+$ (58), 355 (6), 324 (1), 249 (2), 219 (14), 205 (7), 189 (13), 177 (63), 165 (100), 151 (72), 138 (18); 200 MHz ^{1}H NMR ($CDCl_3$): δ3.10 (2H, *m*), 3.85 (6H, *s*), 3.87 (2H, *dd*, J = 3.8, 8.3), 3.88 (6H, *s*), 4.24 (2H, *dd*, J = 6.8, 8.3), 4.74 (2H, *d*, J = 3.8), 6.83–6.89 (6H, *d*, J = 11).

Acknowledgements—This research was supported by grant CA33326 from the Division of Cancer Treatment, National Cancer Institute, National Institute of Health, Bethesda. *In vitro* testing was performed by Dr Linda Jacobsen in the Cell Culture Laboratory, Purdue Cancer Center. High resolution (470 MHz) proton spectra was recorded at the Purdue University Biochemical Magnetic Laboratory (NIH grant RR1077).

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