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Antibacterial and antifungal flavanones from *Eysenhardtia texana*

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Abstract

An activity-guided fractionation of a methanol–dichloromethane extract obtained from the aerial parts of *Eysenhardtia texana* led to the isolation of two novel antibacterial and antifungal flavanones together with a known flavanone. Their structures were established as 4′,5,7-trihydroxy-8-methyl-6-(3-methyl-[2-butenyl])-(2*S*)-flavanone, 4′,5,7-trihydroxy-6-methyl-8-(3-methyl-[2-butenyl])-(2*S*)-flavanone and 4′,5-dihydroxy-7-methoxy-6-(3-methyl-[2-butenyl])-(2*S*)-flavanone on the basis of their UV, 1D and 2D-NMR spectra.

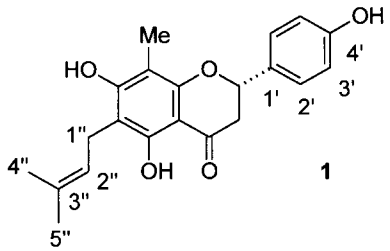
Keywords: *Eysenhardtia texana*; Fabaceae; Prenylated flavanones; Antibacterial; Antifungal

1. Introduction

As part of our ongoing screening of plants from arid regions for possible biomedical uses we observed antifungal and antibacterial properties in the methanol–dichloromethane extract obtained from *Eysenhardtia texana* Kunth. (Fabaceae). This sprawling shrub, which is also regionally known as 'Texas kidneywood', grows on the high calcareous soils of South Texas (north to Bell, San Saba, Crockett and Brewster counties) south to the Mexican states of Coahuila and Tamaulipas. It is usually 2–3 m tall, the leaves are 3–9 cm long with 15–47 mostly 5–15 mm long leaflets (Vines, 1960; Corell & Johhnston, 1970). Bioactivity-guided fractionation using a gel-diffusion assay (Mitscher et al., 1972) for monitoring of the antimicrobial and antifungal activity led to the isolation of two new prenylated flavanones **1** and **2** and the known flavanone **3** (Mahmoud & Waterman, 1985). We here report the structural elucidation of the two new compounds and the determination of their bioactivities.

2. Results and discussion

Maxima of UV absorbance around 290 nm and AMX spin systems in the aliphatic region of the ^{1}H NMR spectra suggested flavanone structures for compounds **1**–**3**. The HRMS of **1** and **2** were in accord-



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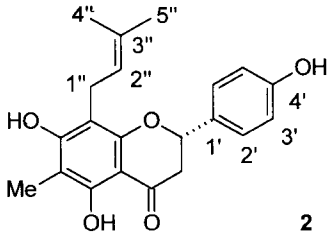

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Table 1
^{13}C and ^{1}H NMR data of compounds **1** and **2** in $CDCl_3$

C/H	**1**		**2**	
	^{13}C	^{1}H	^{13}C	^{1}H
2	78.3 d	5.35 dd (3.0, 12.7)	78.6 d	5.32 dd (3.0, 12.9)
3 ax	43.3 t	3.04 dd (17.1, 12.7)	43.3 t	3.05 dd (17.1, 12.9)
3 eq.	43.3 t	2.81 dd (17.1, 3.0)	43.3 t	2.80 dd (17.1, 3.3)
4	196.5 s	–	196.5 s	–
4a	102.8 s	–	102.7 s	–
5	158.8 s	–	159.8 s	–
6	106.0 s	–	105.1 s	–
7	162.2 s	–	162.0 s	–
8	103.3 s	–	104.4 s	–
8a	158.2 s	–	157.2 s	–
1′	131.2 s	–	131.0 s	–
2′/6′	127.7 d	7.34 AA′BB′ (8.4)	127.7 d	7.33 AA′BB′ (8.4)
4′	155.9 s	–	155.9 s	–
3′/5′	115.6 d	6.89 AA′BB′ (8.4)	115.5 d	6.88 AA′BB′ (8.4)
5-OH	–	12.4 s	–	12.3 s
8- or 6-Me	7.5 q	2.01 s	6.9 q	2.04 s
1″	21.3 t	3.39 br d (7.0)	22.0 t	3.35 br d (7.2)
2″	121.6 d	5.25 br t (7.0)	121.7 d	5.25 br t (7.2)
3″	136.1 s	–	135.7 s	–
4″	17.8 q	1.84 s	17.8 q	1.77 s[a]
5″	25.9 q	1.78 s	25.9 q	1.75 s[a]

[a] Values in column interchangeable.

ance with molecular formulas $C_{21}H_{22}O_5$ (requires: 354.1467, found: 354.1477 and 354.1459). Bathochromic shifts upon addition of $AlCl_3$ and $AlCl_3$/HCl and ^{1}H NMR resonances for chelated hydroxy protons at δ_H 12.4, 12.3 and 12.1 indicated 5-hydroxy substitution. In the aromatic region of the ^{1}H NMR spectra all three compounds showed AA′BB′ spin systems at almost identical positions typical for a 4′-monosubstituted B ring. Molecular ion peaks at *m/z* 354 for **1**–**3** with A ring fragments at *m/z* 234 and B ring fragments at *m/z* 120 indicated a 4′-hydroxylated B ring for all three compounds. Compounds **1** and **2** also showed ^{1}H NMR signals indicative of methyl substituents at an aromatic ring while **3** exhibited a signal typical for a methoxy substituent. Additionally, **1** and **2**, but not **3**, gave bathochromic shifts upon addition of sodium acetate indicating 7-hydroxy (**1**, **2**) and 7-methoxy substitution (**3**). The presence of dimethylallyl groups in **1**–**3** was evidenced by the two singlets for two methyl groups, a doublet for a methylene group and a multiplet for the vinylic proton all at positions typical for dimethylallyl groups attached to aromatic rings. Thus, in **3** one position of the A ring was left unsubstituted as confirmed by a signal at δ_H 6.07. A positive Gibb's test (Horowitz & Gentili, 1964) of **3** indicated an unsubstituted position *para* to the 5-hydroxy group. On the basis of these results and by comparison with published data, **3** was assigned the structure of the known 4′,5-dihydroxy-7-methoxy-6-(3-methyl-[2-butenyl])-(2*S*)-flavanone (Mahmoud & Waterman, 1985).

The positions of the prenyl and methyl groups in **1** and **2** were determined by HMBC experiments. The chelated 5-hydroxy proton of **1** at δ_H 12.4 showed cross peaks with carbons at δ_C 158.8, 106.0 and 102.8 and the prenyl methylene protons at δ_H 3.39 showed cross peaks at δ_C 158.8, 162.2, 136.1, 121.6 and 106.0. Consequently, the position of the prenyl group in **1** is at C-6. The ^{1}H and ^{13}C NMR spectra, DEPT and HMBC experiments together with published data for naringenin (Markham, 1982) also allowed the assignment of carbon and proton shifts for **1** and **2** (Table 1). The proton signal of the methyl group at the A-ring of **2** gave rise to cross peaks with carbons at δ_C 162.0, 159.8 and 105.1 from which its 6-position follows. The levorotatory nature of **1** and **2** indicated normal *S* stereochemistry at C-2. Therefore, we assigned **1** and **2** the structures of 4′,5,7-trihydroxy-8-methyl-6-(3-methyl-[2-butenyl])-(2*S*)-flavanone and 4′,5,7-trihydroxy-6-methyl-8-(3-methyl-[2-butenyl])flavanone, respectively.

At a concentration of 0.1 mg/ml **1** and **2** were shown to inhibit the growth of *Staphylococcus aureus* and **1** also inhibited the growth of *Candida albicans* in an agar-gel diffusion assay. Antibacterial and antifungal activity at this concentration is typical for a variety of prenylated flavonoids described by others (Mitscher, Drake, Gollapudi & Okwute, 1987).

3. Material and methods

3.1. General

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ with TMS as internal standard on a Varian Unity at 300 and 75.4 MHz, respectively. EIMS were recorded on a Hewlett Packard 5988A at 200°C, 70 eV. APCIMS were recorded with a Finnegan TSQ 7000. HREIMS were recorded on a JEOL HX 110 spectrometer with a resolution of 10,000. Optical rotation was determined on a Autopol III polarimeter. A Hitachi HPLC system equipped with a L4500 diode array detector was used for the final purification step. Shifts of UV maxima were measured on a Bausch and Lomb Spectronic 21 spectrophotometer following described procedures (Markham, 1982).

3.2. Determination of biological activity

The agar diffusion assay was carried out as described in the literature (Mitscher et al., 1972) the only modifications being the use of DMSO (total concentration 5%) and SD agar. *S. aureus* and *C. albicans*

from the collection maintained at the Bioresources Research Facility were used.

3.3. Plant material

Aerial parts of *Eysenhardtia texana* Kunth. were obtained from the Desert Legume Program (DELEP), College of Agriculture, University of Arizona. A voucher specimen collected in July, 1990 in Val Verde County, TX, was deposited in the Herbarium of the University of Arizona as ARIZ291230.

3.4. Extraction and isolation

The EtOAc soluble part of 10.9 g CH_2Cl_2–MeOH extract obtained from 150 g dried plant material (aerial parts) was chromatographed with hexane–EtOAc and EtOAc–propan-2-ol mixtures on 600 g silica gel. The resulting fractions were assayed for growth inhibitory activity against *S. aureus* and *C. albicans*. Column chromatography of the most active fr. on Sephadex LH20 and silica gel, prep. TLC and HPLC on RP18 gave 2.8 mg of **1**, 1.5 mg of **2** and 0.6 mg of **3**.

3.5. 4′,5,7-Trihydroxy-8-methyl-6-(3-methyl-[2-butenyl])-(2S)-flavanone (*1*)

$[\alpha]_D$ -5.9° (MeOH, *c* 0.24). UV λ_{max} (nm) MeOH: 296, $AlCl_3$: 320, $AlCl_3$/HCl: 318, NaOAc: 342, NaOAc/H_3BO_3: 296. EIMS *m*/*z* (rel. int.): 354 (46), 339 (10), 311 (10), 299 (20), 234 (24), 219 (52), 206 (44), 191 (36), 179 (100), 120 (38). APCIMS: *m*/*z* 355. ^1H and ^{13}C NMR see Table 1.

3.6. 4′,5,7-Trihydroxy-8-methyl-6-(3-methyl-[2-butenyl])-(2S)-flavanone (*2*)

$[\alpha]_D$ -14.7° (MeOH, *c* 0.14). UV λ_{max} (nm): MeOH: 296, $AlCl_3$: 320, $AlCl_3$/HCl: 318, NaOAc: 336, NaOAc/H_3BO_3: 296. EIMS *m*/*z* (rel. int.): 354 (56), 339 (15), 311(13), 299 (23), 234 (27), 219 (66), 206 (47), 191 (76), 179 (100), 120 (39). APCIMS: *m*/*z* 355. ^1H and ^{13}C NMR see Table 1.

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