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Daucane Sesquiterpenes from *Ferula hermonis*

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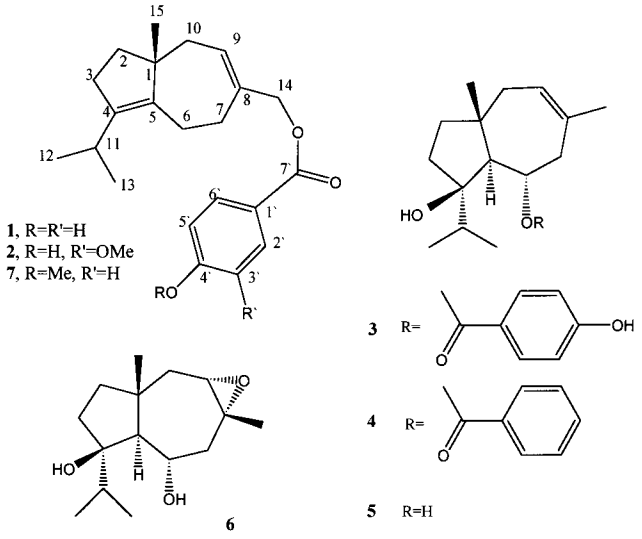
The roots of *Ferula hermonis* Boiss yielded two new daucane esters, 14-(4′-hydroxybenzoyloxy)dauc-4,8-diene (**1**) and 14-(4′-hydroxy-3′-methoxybenzoyloxy)dauc-4,8-diene (**2**), together with the four known sesquiterpenes jaeschkeanadiol *p*-hydroxybenzoate (**3**), jaeschkeanadiol benzoate (**4**), jaeschkeanadiol (**5**), and epoxyjaeschkeanadiol (**6**). The identities of the isolated compounds were ascertained primarily using NMR and MS data. Compounds **1** and **3** exhibited antimicrobial activity against *Staphylococcus aureus* with IC_{50} 1.5 and 3.5 μg/mL, respectively, and against Methicillin-resistant *S. aureus* with IC_{50} 2.0 and 4.0 μg/mL, respectively.

Ferula (Umbelliferae) is a large genus of about 150 species.[1] Some species of *Ferula* have been used in traditional medicine for skin infections,[2] fever,[2] dysentry,[3] and as an antihysteric.[3] *Ferula hermonis* is also used in the Middle East as a potential aphrodisiac. Many daucane sesquiterpenoids with diverse oxygenation patterns have been isolated from different species of *Ferula*.[4−8] Previous phytochemical investigation on *F. hermonis* resulted in the isolation of the 8,9-epoxy derivative of the daucane sesquiterpene jaeschkeanadiol benzoate together with two other known sesquiterpenes: the less frequently occurring (+)-α-bisabolol and jaeschkeanadiol vanillate.[9]

The dried and ground roots of *Ferula hermonis* Boiss (Umbelliferae) were extracted with hexane, and the extract was partitioned between MeCN and hexane. The MeCN fraction was subjected to repeated silica gel column chromatography to afford **1** and **2**.

The ^{1}H and ^{13}C NMR spectra of **1** (Experimental Section) exhibited close similarity to those of 14-(4′-anisoyloxy)dauc-4,8-diene (**7**),[10] except for the presence of a 4′-hydroxybenzoyl moiety instead of a 4′-anisoyl moiety at C-14. The occurrence of three methyl groups was indicated by the ^{1}H NMR signals at δ 0.93 (3H, d, J = 6.9 Hz, Me-12), δ 0.98 (3H, d, J = 7.0 Hz, Me-13), and δ 0.95 (3H, s, Me-15), and at δ 21.2 (C-12), δ 21.8 (C-13), and δ 23.4 (C-15) in the ^{13}C NMR spectrum. ^{13}C NMR also showed the existence of an ester carbonyl and three quaternary carbon signals at δ 166.7, 138.1, 139.4, and 141.3, respectively, and one methine signal at δ 128.5. These carbon signals were similar to those of structurally related compounds,[7,8] which proved the existence of a double bond between C-4 and C-5 and another one between C-8 and C-9. The ^{1}H NMR supported the presence of a methine proton at δ 5.85 (H-9). The appearance of a signal at δ 4.69 (H-14) in the ^{1}H NMR and at δ 70.4 in the ^{13}C NMR were evidence for the presence of an allylic methylene group (C-14) and the attachment of the acyl moiety to this allylic methylene. High-resolution electrospray ionization Fourier transform MS (HRESIFTMS) of **1** showed an ion peak for $[M - H]^+$ at *m/z* 339.1961, in accordance with a molecular formula of $C_{22}H_{28}O_3$. The IR spectrum supported the presence of an ester carbonyl and a free hydroxyl group at 1713 and 3357 cm^{-1}, respectively. Thus, the structure of **1** was established as 14-(4′-hydroxybenzoyloxy)dauc-4,8-diene.



The spectral data of **2** clearly indicated that it is a 3′-methoxy derivative of **1**. Signals were present at δ 3.92 (OMe) in the ^{1}H NMR and at δ 56.5 in the ^{13}C NMR. The IR spectrum of **2** provided evidence for a free hydroxyl group with a strong absorption band at 3395 cm^{-1}. The placement of the hydroxyl group at position 4′ and the methoxy group at position 3′ in the benzoyl moiety was based on HMBC and NOESY experiments (Figure 1), which confirmed the identity of **2** as 14-(4′-hydroxy-3′-methoxybenzoyloxy)dauc-4,8-diene. Compounds **3**, **4**, **5**, and **6** have previously been isolated from other natural sources, and their structures were identified by comparison of their spectral data with literature values.[8,10,11]

Compounds **1** and **3** exhibited antimicrobial activity against *Staphylococcus aureus* with IC_{50} 1.5 and 3.5 μg/mL, respectively, and against Methicillin-resistant *S. aureus* with IC_{50} 2.0 and 4.0 μg/mL, respectively. The MIC in all cases was 6.25 μg/mL. Compounds **2**, **4**, **5**, and **6** were inactive.

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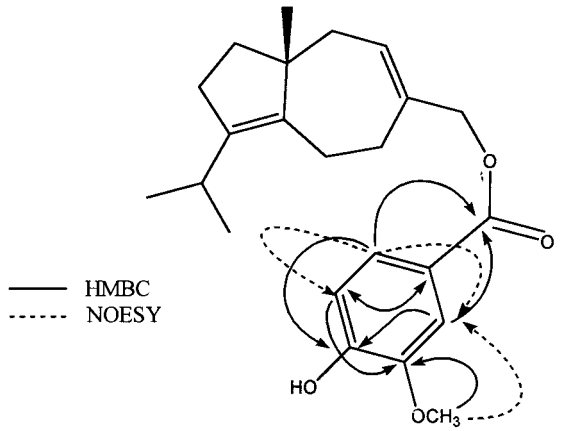

Figure 1. Important HMBC and NOESY interactions in compound **2**.

Experimental Section

General Experimental Procedures. IR spectra were recorded with an ATI Mattson Genesis Series Fourier transform (FT-IR) spectrophotometer. UV spectra were obtained on a Hewlett-Packard 8452A diode array spectrophotometer. Optical rotations were recorded at ambient temperature using a Jasco DIP-370 digital polarimeter. 1D and 2D NMR spectra were obtained on Bruker Avance DRX 400 and 500 spectrometers. HRESIFTMS was obtained using a Bruker Bioapex FT-MS in ESI mode. Low-resolution MS was measured on a ThermoQuest aQa LC/MS. For TLC, glass-supported silica gel 60 plates (0.25 mm layer, F_{254}, E. Merck) were used. Visualization was accomplished by spraying with *p*-anisaldehyde spray reagent followed by heating at 110 °C. Silica gel 60 (230−400 mesh, E. Merck) was used for column chromatography.

Plant Material. The dried roots of *Ferula hermonis* Boiss were purchased from herbal stores in Syria and identified by Dr. Sultan-ul-Abdeen, College of Pharmacy, King Saud University. A voucher specimen (No. 1407) is deposited at the herbarium of the College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia.

Extraction and Isolation. The dried and ground roots of *Ferula hermonis* Boiss (20 g) were exhaustively extracted with hexane in a continuous extraction apparatus. The hexane extract (3.76 g) was partitioned between MeCN and hexane. The MeCN fraction (3.38 g) was subjected to silica gel column chromatography (120 g) using 10% to 40% EtOAc in hexane as eluent (20 mL fractions). Fractions 99−115, eluted with 20% EtOAc in hexane, were pooled and concentrated in vacuo. The oily residue (49.2 mg) was rechromatographed on a silica gel column (30 g) and eluted with 4% MeCN in CH_2Cl_2, and 50 fractions were collected. Fractions 19−20 yielded compound **1** (5.5 mg, oil, R_f 0.42, system: CH_2Cl_2−MeCN, 96:4). Fractions 222−233, eluted from the first column with 30% EtOAc in hexane, were also pooled and concentrated in vacuo to an oily residue (29 mg), which was further purified on a silica gel column (2 g), using CH_2Cl_2 for elution. This column afforded compound **2** (5.0 mg, oil, R_f 0.58, system: CH_2Cl_2−MeCN, 96:4).

14-(4′-Hydroxybenzoyloxy)dauc-4,8-diene (1): oil, $[\alpha]_D$ +13.9 (*c* 0.03, MeOH); UV λ_{max} (log ϵ) (MeOH) 286 (2.67) nm; IR (film) ν_{max} 3357 (OH), 2956, 2931, 1713 (C=O), 1608, 1592, 1514, 1545, 1374, 1278 cm $^{-1}$; ^{1}H NMR ($CDCl_3$) δ 0.93 (3H, d, J = 6.9 Hz, Me-12), 0.95 (3H, s, Me-15), 0.98 (3H, d, J = 7.0 Hz, Me-13), 2.06 (1H, m, H-10), 2.19 (1H, m, H-10′), 2.66 (1H, septet, J = 6.8 Hz, H-11), 4.69 (2H, d, J = 13.0 Hz, H-14), 5.85 (1H, br t, J = 6.2 Hz, H-9), 6.33 (1H, s, 4′-OH), 6.88 (2H, d, J = 8.6 Hz, H-3′, H-5′), 7.96 (2H, d, J = 8.0 Hz, H-2′, H-6′); ^{13}C NMR ($CDCl_3$) δ 49.2 (s, C-1), 38.6 (t, C-2), 29.8 (t, C-3), 141.3 (s, C-4),139.4 (s, C-5), 22.8 (t, C-6), 27.1 (t, C-7), 138.1 (s, C-8), 128.5 (d, C-9), 40.3 (t, C-10), 26.4 (d, C-11), 21.2 (q, C-12), 21.8 (q, C-13), 70.4 (t, C-14), 23.4 (q, C-15), 122.6 (s, C-1′), 131.9 (d, C-2′, C-6′), 115.2 (d, C-3′,C-5′), 160.2 (s, C-4′), 166.7 (s, C-7′); HRESIFTMS *m/z* 339.1961 [M − H]$^-$ (calcd for $C_{22}H_{29}O_3$, 339.1955).

14-(4′-Hydroxy-3′-methoxybenzoyloxy)dauc-4,8-diene (2): oil, $[\alpha]_D$ −13.0 (*c* 0.052, MeOH); UV λ_{max} (log ϵ) (MeOH) 302 (3.27) nm; IR (film) ν_{max} 3394 (OH), 2955, 1711 (C=O), 1597, 1514, 1462, 1429, 1378, 1282 1216 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.92 (3H, d, J = 6.8 Hz, Me-12), 0.95 (3H,s, Me-15), 0.98 (3H, d, J = 6.8 Hz, Me-13), 2.06 (1H, m, H-10), 2.18 (1H, m, H-10′), 2.66 (1H, septet, J = 6.8 Hz, H-11), 3.92 (3H, s, OCH_3), 4.70 (2H, br s, H-14), 5.87 (1H, br t, J = 9.8 Hz, H-9), 6.93 (1H, d, J = 8.3 Hz, H-5′), 7.64 (1H, dd, J = 8.3, 1.7 Hz, H-6′), 7.56 (1H, d, J = 1.8 Hz, H-2′), 6.15 (1H, s, 4′-OH); ^{13}C NMR ($CDCl_3$) δ 49.2 (s, C-1), 39.1 (t, C-2), 30.2 (t, C-3), 141.3 (s, C-4), 139.4 (s, C-5), 23.2 (t, C-6), 27.5 (t, C-7), 138.4 (s, C-8), 128.6 (d, C-9), 40.7 (t, C-10), 26.8 (d, C-11), 21.6 (q, C-12), 22.3 (q, C-13), 70.7 (t, C-14), 23.6 (q, C-15), 122.5 (s, C-1′), 114.5 (d, C-2′), 146.2 (s, C-3′), 150.0 (s, C-4′), 112.2 (d, C-5′), 124.5 (d, C-6′), 166.3 (s, C-7′), 56.5 (s, OCH_3); HRESIFTMS *m/z* 369.2080 [M − H]$^-$ (calcd for $C_{23}H_{31}O_4$, 369.2060).

Antimicrobial Bioassay. Three opportunistic fungal strains (*Candida albicans* ATCC 90028, *Cryptococcus neoformans* ATCC 90113, and *Aspergillus fumigatus* ATCC 90906) and four pathogenic bacteria (*Staphylococcus aureus* ATCC 29213, methicillin-resistant *S. aureus* ATCC 43300 (MRS), *Pseudomonas aeruginosa* ATCC 27853, *and Mycobacterium intracellulare* ATCC 23068) were used in the in vitro evaluation of the isolated compounds. Susceptibility testing was performed using a modified version of the NCCLS methods.[12] The microbial inocula, excluding *A. fumigatus*, were prepared by diluting the subcultured organism in its incubation broth. The *A. fumigatus* inoculum was prepared by gently removing the growth from a slant and transferring to 50 mL of YPD broth. Prepared test compounds/extracts were dissolved in DMSO, serially diluted using normal saline, and transferred in duplicates to 96-well microtiter plates. The microbial inoculum was added to achieve a final volume of 200 μL and final concentrations starting with 500 μg/mL for crude extracts and 50 μg/mL for pure compounds. Drug [tetracycline (Sigma, St. Louis, MO) for bacteria and amphotericin B (ICN Biomedicals, OH) for fungi] as well as growth and blank (media only) controls were added to each test plate. Except for *M. intracellulare* and *A. fumigatus*, which were inspected visually, all other organisms were read turbidimetrically at 630 nm using the EL-340 Biokinetics Reader (Bio-Tek Instruments, Vermont) prior to and after incubation. For turbidimetrically read organisms, percent growth was calculated and plotted versus concentration to afford the IC_{50}/MIC. Minimum fungicidal or bactericidal concentrations (MFC/MBC) were determined by removing 5 μL of each duplicate, transferring to agar, and incubating at previously mentioned times and temperatures.

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