Furanosesquiterpenoids of *Commiphora myrrha*

Nanqun Zhu,[†] Hiroe Kikuzaki,[‡] Shuqun Sheng,[§] Shengmin Sang,[†] Mohamed M. Rafi,[†,⊥] Mingfu Wang,[†] Nobuji Nakatani,[‡] Robert S. DiPaola,[⊥] Robert T. Rosen,[†] and Chi-Tang Ho*[,†]

Department of Food Science, Rutgers University, New Brunswick, New Jersey 08901, Graduate School of Human Life Science, Osaka City University, Sumiyoshi, Osaka, Japan, Department of Chemistry, Rutgers University, Piscataway, New Jersey 08854, and Cancer Institute of New Jersey, University of Medicine and Dentistry of New Jersey, New Brunswick, New Jersey 08901

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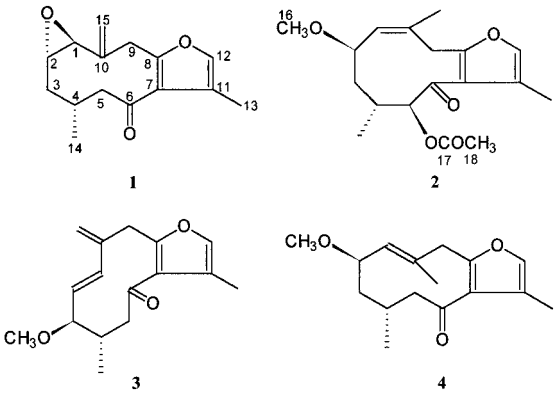
An investigation on the gum exudates of *Commiphora myrrha* has led to the isolation of six sesquiterpenoids. On the basis of spectroscopic data interpretation, they were determined as two new furanosesquiterpenoids, *rel*-1*S*,2*S*-epoxy-4*R*-furanogermacr-10(15)-en-6-one (**1**) and *rel*-2*R*-methyl-5*S*-acetoxy-4*R*-furanogermacr-1(10)*Z*-en-6-one (**2**), and four known furanosesquiterpenoids, *rel*-3*R*-methoxy-4*S*-furanogermacra-1*E*,10(15)-dien-6-one(**3**),*rel*-2*R*-methoxy-4*R*-furanogermacr-1(10)*E*-en-6-one(**4**),furanogermacra-1(10)*Z*,4*Z*-dien-6-one, and curzerenone [6,7-dihydro-5β-isopropenyl-3,6β-dimethyl-6-vinylbenzofuran-4(5H)-one]. This is the first report of the relative stereochemistry for the known compounds **3** and **4**. Compound **1** exhibited weak cytotoxic activity against a MCF-7 breast tumor cell line in a clonogenic assay, while the other five compounds were inactive in this assay.

As a plant found in some African and Asian countries, such as Kenya, India, and People's Republic of China, *Commiphora myrrha* Engl. (Burseraceae) yields a yellow nonvolatile gum resin (myrrh), which has been used as a food additive, a fragrance, and a traditional medicine to treat stomach complaints and diarrhea.[1] Gums of certain *Commiphora* species are often combined as commercial samples used in industry.[1] There have been several investigations to show that the essential oils of *Commiphora* species are rich in furanosesquiterpenoids, and a total of around 20 different compounds of this type have been identified from plants in this genus.[1−6] These isolated furanosesquiterpenoids or crude extracts of C*ommiphora* gum have been found to possess anaesthetic, antibacterial, antifungal, and antihyperglycemic properties.[6−8] However, limited chemical studies have been done on *Commiphora myrrha* collected in mainland China.[9] Here, we report the isolation of two new furanosesquiterpenoids (**1** and **2**), as well as four known derivatives from a Chinese sample of *C. myrrha*. The structures of the new compounds **1** and **2** were elucidated by spectroscopic methods and with the aid of molecular modeling. The identities of the four known compounds, *rel*-3*R*-methoxy-4*S*-furanogermacra-1*E*,10(15)-dien-6-one (**3**), *rel*-2*R*-methoxy-4*R*-furanogermacr-1(10)*E*-en-6-one (**4**), furanogermacra-1(10)*Z*,4*Z*-dien-6-one, and curzerenone [6,7-dihydro-5β-isopropenyl-3,6β-dimethyl-6-vinylbenzofuran-4(5H)-one], were established by comparison with published data.[2,3,5]

Compound **1** was obtained as a colorless oil which showed a protonated molecular ion $[M + 1]^+$ at *m*/*z* 247 in the positive-ion APCIMS. Together with its ^{13}C NMR spectrum (Table 1) and elemental analysis, the molecular formula was determined as $C_{15}H_{18}O_3$, which suggested it is a sesquiterpenoid with seven degrees of unsaturation.

The ^1H NMR spectrum of **1** (Table) showed a characteristic downfield signal at δ 7.04 (1H, q, $J = 1.2$ Hz) and a doublet at δ 2.13 (3H, d, $J = 1.2$ Hz), which were assigned as the α-proton and the β-methyl of a furan ring, respectively. By comparison of chemical shifts in the ^{13}C NMR spectrum of **1** with literature data[2] and with the help of HMQC and HMBC spectra, the chemical shifts of the furan carbons (C-7, C-8, C-11, C-12) were assigned at δ 123.3, 157.1, 118.0, and 138.5, respectively. Based on the multiplicity shown in the HMQC spectrum, the remaining two downfield signals at δ 110.3 and 141.4 belonged to one 1,1-disubstituted double bond. The signal at δ 200.7 was assigned to a carbonyl group.



The structure of **1** was determined by a detailed analysis of its 1D and 2D NMR spectra. The HMQC spectrum permitted the assignment of all protons to the corresponding carbon atoms as shown in Table 1, and $^1H-^1H$ COSY and HMBC correlations permitted the connectivity to be established for this 10-membered ring. Compared with previously known structures, the difference was the occurrence of a 1,2-epoxide ring in **1**, which was assembled by the following evidence. In the $^1H-^1H$ COSY spectrum, the signal for H-2 at δ 1.92 (1H, ddd $J = 2.2, 2.2, 10.3$ Hz) coupled with signals for H-3 at δ 1.09 (1H, ddd, $J = 10.3, 10.3, 13.9$ Hz) and 2.10 (1H, br d, $J = 13.9$ Hz). In the HMBC spectrum (Figure 1), H-1 correlated with C-2, C-3, C-10, and C-15; H-2 correlated with C-3; and H-15 correlated with C-1. In view of the degree of unsaturation and the chemical shifts of C-1 (59.7 ppm) and C-2 (61.4 ppm), the planar structure of **1** could be determined.

* To whom correspondence should be addressed. Tel: 732-932-9611, ext 235. Fax: 732-932-6776. E-mail: ho@aesop.rutgers.edu.
[†] Department of Food Science, Rutgers University.
[‡] Osaka City University.
[§] Department of Chemistry, Rutgers University.
[⊥] University of Medicine and Dentistry of New Jersey.

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Table 1. NMR Data of **1** and **2**

	1		**2**	
position	^{1}H	^{13}C	^{1}H	^{13}C
1	2.96 (1H, brs)	59.7 d	5.20 (1H, brd, J = 7.9 Hz)	130.1 d
2	1.92 (1H, ddd, J = 10.3, 2.2, 2.2 Hz)	61.4 d	4.35 (1H, m)	76.9 d
3	2.10 (1H, brd, J = 13.9 Hz, H-3a)	39.9 t	1.29 (1H, m, H-3a)	39.6 t
	1.09 (1H, ddd, J = 10.3, 10.3, 13.9 Hz, H-3b)		1.98 (1H, m, H-3b)	
4	2.18 (1H, m)	29.3 d	2.16 (1H, m)	32.0 d
5	2.96 (1H, ddd, J = 1.5, 3.5, 13.9 Hz, H-5a)	53.4 t	5.87 (1H, brs)	76.7 d
	2.31 (1H, dd, J = 12.0, 13.9 Hz, H-5b)			
6		200.7 s		192.1 s
7		123.3 s		123.9 s
8		157.1 s		157.5 s
9	3.47 (1H, d, J = 14.2 Hz, H-9a)	32.5 t	3.35 (1H, d, J = 14.2 Hz, H-9a)	34.7 t
	4.18 (1H, brd, J = 14.2 Hz, H-9b)		4.06 (1H, d, J = 14.2 Hz, H-9b)	
10		141.4 s		120.2 s
11		118.0 s		118.3 s
12	7.04 (1H, q, J = 1.2 Hz)	138.5 d	7.11 (1H, brs)	139.5 d
13	2.13 (3H, d, J = 1.2 Hz)	10.1 q	2.17 (3H, brs)	10.9 q
14	1.12 (3H, d, J = 6.8 Hz)	24.1 q	0.93 (3H, d, J = 6.5 Hz)	14.6 q
15	4.95 (2H, brs)	110.3 t	1.86 (3H, brs)	24.3 q
16			3.33 (3H, s)	57.3 q
17				171.7 s
18			2.24 (3H, s)	21.1 q

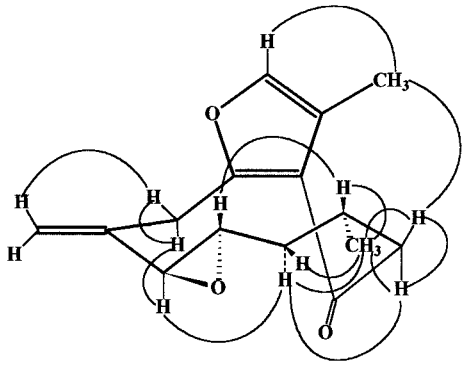

Figure 1. Key NOESY correlations for compound **1**.

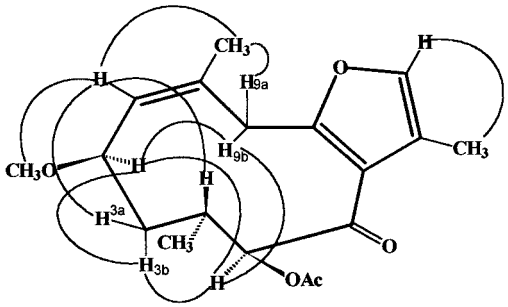

Figure 2. Key NOESY correlations for compound **2**.

With the help of a Dreiding molecular model, the relative stereochemistry of **1** was suggested by the NOESY correlations shown in Figure 1, in which diagnostic correlation contours between H-1 and H-3b, H-1 and H-9b, H-2 and H-4, H-3 and H-14, H-3b and H-5b, and H-5a and H-13 were observed. As a result of this stereochemistry, the deshielding effect of the carbonyl group caused the relatively downfield shift of H-9b (4.18 ppm), while the extremely high-field shift of H-2 (1.92 ppm) was due to the shielding effect of the furano group. Thus, compound **1** was deduced as *rel*-1*S*,2*S*-epoxy-4*R*-furanogermacr-10(15)-en-6-one.

Compound **2** was obtained as a colorless oil with the molecular formula $C_{18}H_{24}O_5$ based on the ^{13}C NMR spectrum, elemental analysis, and the positive-ion APCIMS, which showed a protonated molecular ion $[M + 1]^+$ at *m*/*z* 321. Similar to **1**, **2** was determined as a furanosesquiterpenoid based on the germacrane skeleton with the addition of one methoxyl group and one acetoxyl group. In the ^{1}H NMR spectrum, the α-proton signal of the furan ring appeared at δ 7.11 (1H, br s) and the β-methyl signal could be located at δ 2.17 (3H, br s). The chemical shifts of the furan carbons (C-7, C-8, C-11, C-12) were assigned at δ 118.3, 157.5, 123.9, and 139.5, respectively. Assignments of C-7 and C-11 were suggested by the HMBC correlation of between H-9 and C-7.

Following the same NMR procedures used for **1**, the locations of the methoxyl and acetoxyl groups were determined by the following key ^{1}H−^{1}H COSY and HMBC correlations: H-1 with H-2; H-2 with H-3; H-2 with C-3 and C-16; H-5 with C-3, C-4, C-6, C-14, and C-17; H-18 with C-17. On the basis of all of this evidence, the planar structure of **2** was found to be the same as that reported by Brieskorn and Noble for a compound isolated from *Commiphora molmol*.[2] However, comparison of the spectral data (in $CDCl_3$ of both cases), in particular those for the H-5 and H-9 signals, suggested that **2** has a different stereochemistry. In the ^{1}H NMR spectrum, signals at δ 3.35 (d, J = 14.2 Hz), 4.06 (d, J = 14.2 Hz), and 5.87 (br s) were assigned to H-9a, H-9b, and H-5 of compound **2**, respectively, while H-9a, H-9b, and H-5 of the known compound were assigned at δ 3.30 (d, J = 16.5 Hz), 3.66 (d, J = 16.5 Hz), and 5.57 (br d, J = 8.5 Hz), respectively.[2]

With the help of a Dreiding molecular model, the relative stereochemistry of **2** was suggested by the NOESY correlations shown in Figure 2. In the NOESY spectrum, key correlation contours (H-1 with H-3a, H-4, and H-15; H-2 with H-3b, H-5, and H-9b; H-5 with H-2 and H-9b) were observed. In addition, almost no proton−proton coupling between H-4 and H-5 was apparent, thereby indicating that H-4 is at right angles to H-5. On the basis of these spectroscopic studies, compound **2** was deduced to be *rel*-2*R*-methyl-5*S*-acetoxy-4*R*-furanogermacr-1(10)*Z*-en-6-one (**2**).

In addition, with the help of NOESY spectra and Dreiding molecular models, the relative stereochemistry of known compounds **3** and **4** were determined for the first time. For **3**, NOESY correlations (H-3 with H-1, H-5, and H-14; H-2 with H-4; H-5 with H-13 and H-14) indicated that the methoxyl group on C-3 and the methyl group on C-4 are *trans* and both are quasi-equatorially oriented, and the conformation of **3** is as shown. For **4**, NOESY correla-

tions between H-2 and H-15, H-2 and H-14, H-2 and H-3, H-1 and the methoxyl group on C-2, and H-3 and H-4 suggested the relative stereochemistry of **4** to be as shown.

In a clonogenic assay following known protocols,[10] compound **1** showed a weak inhibitory activity against a MCF-7 breast cell line with an IC_{50} of 40 μM, while the other five compounds obtained in this study were inactive.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 141 polarimeter at 25 °C. IR spectra were recorded on a Nicolet 5000 infrared spectrometer. NMR spectra of compound **1** were recorded on a Varian U-500 instrument, and the other NMR spectra were obtained on a Varian U-400 instrument with TMS as internal reference and $CDCl_3$ as solvent. Signals are reported in ppm (δ). Elemental compositions were analyzed on a Carlo Erba 1106 instrument. Positive APCIMS were obtained on a Micromass Platform II system (Micromass, Beverly, MA) equipped with a Digital DECPC XL560 computer for data analysis. TLC was performed on Sigma-Aldrich Si gel TLC plates (250 μm thickness, 2−25 μm particle size), with compounds visualized by UV_{365nm} light and spraying with 10% (v/v) H_2SO_4 ethanol solution. For chromatography, Si gel (130−270 mesh) and reversed-phase C_{18} Si gel (RP-18) (60 μm) (Sigma Chemical Co., St. Louis, MO) were used. All solvents used were purchased from Fisher Scientific (Springfield, NJ).

Plant Material. The exudate of *Commiphora myrrha* was purchased from Meixing Co. (Edison, NJ) in May 1999, which was collected in Weicheng County, Heinan Province, People's Republic of China, and imported from Shanghai Chinese Medicinal Herbs Co., People's Republic of China. It was identified by Professor Zhi-wei Wang, Department of Pharmacognosy, Shanghai Medical University, People's Republic of China. A specimen of this plant material (No. CM9905) has been deposited at the herbarium of the Food Science Department, Rutgers University.

Extraction and Isolation. *Commiphora myrrha* exudates (2.0 kg) were extracted with ethyl acetate at room temperature for one week. The residue was filtered, and the ethyl acetate extracts were combined and concentrated under reduced pressure (650 g). A sample (100 g) was directly subjected to column chromatography on Si gel and eluted with a gradient mixture of chloroform and methanol with increasing methanol content, and nine fractions (A) were collected. Fraction A1 was subjected to Si gel column chromatography with hexanes−ethyl acetate (50:1) as the eluent, to give four subfractions (B). Subfraction B3 was further purified on a RP-18 column, using methanol−water (2.3:1) for elution, to afford **1** (85 mg) and curzerenone [6,7-dihydro-5β-isopropenyl-3,6β-dimethyl-6-vinylbenzofuran-4(5H)-one] (60 mg). Fraction A2 was eluted with hexanes−ethyl acetate (50:1) over Si gel to give five subfractions (C1−C5), while further purification of subfractions C2 and C4 on a RP-18 column, using methanol−water (2.6:1) as the eluent, afforded furanogermacra-1(10)*Z*,4*Z*-dien-6-one (16 mg), **3** (25 mg), and **4** (65 mg). Fraction A3 was rechromatographed on an RP-18 column with methanol−water (3:1) to give compound **2** (33 mg). The four known compounds, *rel*-3*R*-methoxy-4*S*-furanogermacra-1*E*,10(15)-dien-6-one (**3**), *rel*-2*R*-methoxy-4*R*-furanogermacr-1(10)*E*-en-6-one (**4**), furanogermacra-1(10)*Z*,4*Z*-dien-6-one, and curzerenone [6,7-dihydro-5β-isopropenyl-3,6β-dimethyl-6-vinylbenzofuran-4(5H)-one], exhibited spectral data consistent with literature values.[2,3,5]

***rel*-1*S*,2*S*-Epoxy-4*R*-furanogermacr-10(15)-en-6-one (1):** colorless oil; $[\alpha]_D$ −160.0° (*c* 2.5, $CHCl_3$); IR (KBr) ν_{max} 1650, 1239 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; positive-ion APCIMS *m*/*z* 229 $[M + 1]^+$; *anal*. C 73.02%, H 7.39%, calcd for $C_{15}H_{18}O_3$, C 73.15%, H 7.37%.

***rel*-2*R*-Methyl-5*S*-acetoxy-4*R*-furanogermacr-1(10)*Z*-en-6-one (2):** colorless oil; $[\alpha]_D$ +113.6° (*c* 2.3, $CHCl_3$); IR (KBr) ν_{max} 17 41, 1237 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; positive-ion APCIMS *m*/*z* 321 $[M + 1]^+$; *anal*. C 67.11%, H 7.63%, calcd for $C_{18}H_{24}O_5$, C 67.48%, H 7.55%.

***rel*-3*R*-Methoxy-4*S*-furanogermacra-1*E*,10(15)-dien-6-one (3):** colorless oil; $[\alpha]_D$ 74.4°(*c* 0.80, $CHCl_3$); positive-ion APCIMS *m*/*z* 261 $[M + 1]^+$; exhibited spectral data consistent with literature values.[2]

***rel*-2*R*-Methoxy-4*R*-furanogermacra-1(10)*E*-en-6-one (4):** crystal from chloroform; $[\alpha]_D$ −174.0° (*c* 1.0, $CHCl_3$); positive-ion APCIMS *m*/*z* 263 $[M + 1]^+$; exhibited spectral data consistent with literature values.[2]

Furanogermacr-1(10)*Z*,4*Z*-dien-6-one: $[\alpha]_D$ −9.8° (*c* 0.5, $CHCl_3$); exhibited spectral data consistent with literature values.[3]

Curzerenone [6,7-dihydro-5β-isopropenyl-3,6β-dimethyl-6-vinylbenzofuran-4(5H)-one]: $[\alpha]_D$ 0.7° (*c* 0.3, $CHCl_3$); exhibited spectral data consistent with literature values.[5]

Cell Culture. MCF-7 breast tumor cells obtained from American Type Culture Collection (ATCC, Rockville, MD) were cultured in Roswell Park Memorial Institute-1640 supplemented with 10% fetal bovine serum (FBS), 50 units penicillin, and 50 μg/mL streptomycin. Cells were routinely checked and found to be free of contamination by mycoplasma.

Clonogenic Assay. This method was adapted from that of Sullivan et al.[10] MCF-7 cells were plated at 5×10^2 cells/mL in 60 × 15 mm tissue culture dishes with different concentrations of test agents. After 15 days, the medium was aspirated and plates were incubated for 3 min in methylene blue (2.5 g of methylene blue trihydrate, 250 mL water, and 250 mL 95% ethanol). The stain was removed and plates were rinsed in tepid running water. When dry, colonies were counted using the Quantity One software (Bio-Rad Laboratories, Inc., Hercules, CA). The IC_{50} values were obtained by extrapolation from linear regression analysis.

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