Myrrhanolide D and Myrrhasin A, New Germacrane-Type Sesquiterpenoids from the Resin of *Commiphora opobalsamum*

by **Tao Shen**[a]), **Guo-Hui Li**[b]), **Qing-Qing Zhong**[c]), **Shu-Qi Wang**[a]), **Dong-Mei Ren**[a]), **Hong-Xiang Lou***[a]), and **Xiao-Ning Wang***[a])

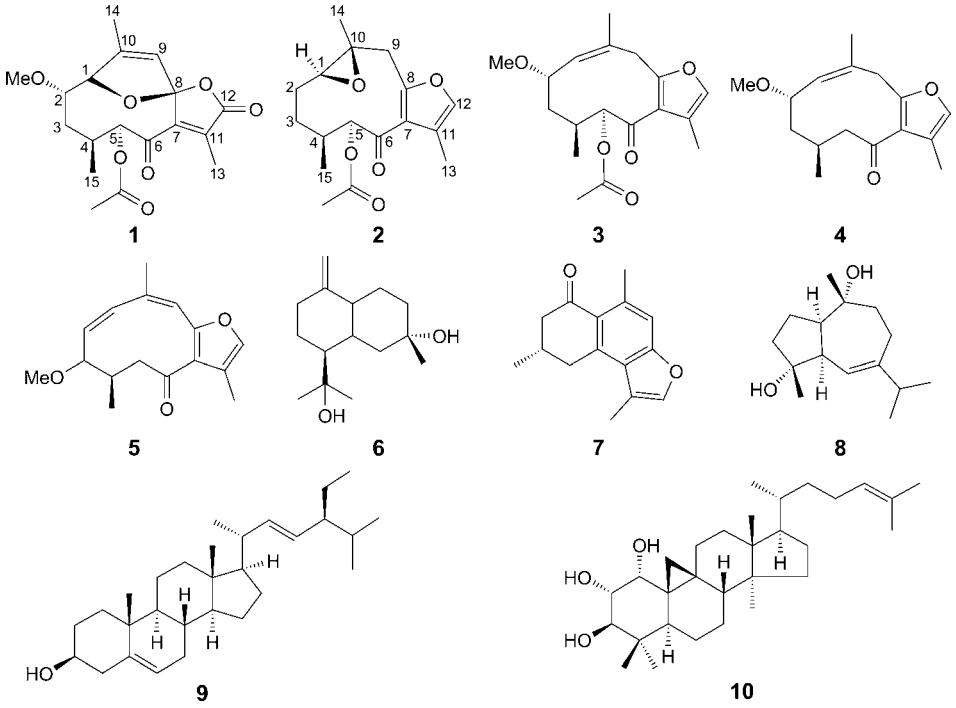
[a]) Key Lab of Chemical Biology (MOE), School of Pharmaceutical Sciences, Shandong University, Jinan, P. R. China (phone: +86-531-88382012; fax: +86-531-88382548; e-mail: wangxn@sdu.edu.cn)
[b]) Department of Pharmacy, Jinan Maternity and Child Care Hospital, Jinan, P. R. China
[c]) School of Pharmaceutical Sciences, Shandong University of Traditional Chinese Medicine, Jinan, P. R. China

Two new germacrane-type sesquiterpenoids bearing an epoxy ring, myrrhanolide D (**1**) and myrrhasin A (**2**), together with eight known compounds, **3**–**10**, were isolated from the resinous exudates of *Commiphora opobalsamum*. Their structures were elucidated based on the analyses of their spectroscopic data. The isolated compounds **1**, **2**, **6**, and **8** were evaluated for their cytotoxic activities against human prostate cancer DU145 and PC3 cells.

Introduction. – The genus *Commiphora* of the Burseraceae family comprises over 150 species, and mainly occurs in Eastern Africa, Arabia, and India [1]. The resinous exudates of plants from this genus are used in indigenous medicines for the treatment of wounds, pain, arthritis, fractures, obesity, parasitic infection, and gastrointestinal diseases. Many metabolites, including terpenoids, steroids, flavonoids, lignans, and carbohydrates, have been discovered in this genus [2][3]. Isolated ingredients and crude extracts exhibited diverse biological features, such as anti-inflammatory, analgesic, cytotoxic, antimicrobial, cardiac protection, and hepatoprotective activities [1][2][4][5].

In our systematic investigation of chemical constituents from the genus *Commiphora*, *ca.* 50 secondary metabolites, including 23 new compounds, have been reported from three *Commiphora* species [6–10]. Motivated by the recent discovery of new natural products from this genus [11–14], we reinvestigated the resinous exudates of *C. opobalsamum*, and isolated two new germacrane-type sesquiterpenoids, **1** and **2**, together with eight known compounds **3**–**10**. Compounds **1**, **2**, **6**, and **8** were evaluated for their cytotoxic activities against human prostate cancer DU145 and PC3 cells.

Results and Discussion. – Myrrhanolide D (**1**), obtained as yellow oil, exhibited a *pseudo*-molecular-ion peak at *m*/*z* 373.2 ($[M+Na]^+$) in the positive-ion mode ESI-MS, consistent with the molecular formula $C_{18}H_{22}O_7$ deduced from the HR-ESI-MS *m*/*z* 351.1454 ($[M+H]^+$; calc. 351.1444). The ^{13}C-NMR displayed 18 signals (*Table*), which were classified to correspond to five Me groups, one sp^3 CH_2 group, four sp^3 CH groups (three oxygenated (δ(C) 76.3, 78.5, and 88.6)), one quaternary C-atom, two C=C bonds, and three C=O groups (δ(C) 169.3, 170.2, and 199.6), based on the chemical



shifts and HMQC correlations. The ^{1}H-NMR spectrum (*Table*) revealed the presence of four tertiary Me groups (δ(H) 1.86, 1.87, 2.08, and 3.41), a secondary Me group (δ(H) 1.16 (d, $J = 6.0$)), one olefinic H-atom (δ(H) 5.41), and three CH–O groups H-atoms geminal to O-atoms (δ(H) 3.76, 5.21, and 5.10). These NMR data suggested that **1** might be a germacrane-type sesquiterpenoid with a MeO group (δ(H) 3.41; δ(C) 57.4) and an AcO group (δ(H) 2.08; δ(C) 20.1, 170.2), which was established by the analysis of its 2D-NMR spectra. The ^{1}H,^{1}H-COSY spectrum led to the establishment of structural fragments as depicted with bold lines in *Fig. 1*. Interpretation of long-range HMBCs Me(13)/C(7), C(11), and C(12); Me(14)/C(1), C(9), and C(10); H–C(9)/C(8); Me(15)/C(3), C(4), and C(5); and H–C(5)/C(6) (*Fig. 1*) indicated that **1** possessed the same skeleton as the known sesquiterpenoid 1β,8β-epoxy-2α-methoxy-6-oxogermacra-9,7(11)-dien-8,12-olide [11]. The positions of the MeO group at C(2) and of the AcO group at C(5) were deduced from the cross-peaks H–C(2)/δ(C) 57.4 (MeO) and H–C(5)/δ(C) 170.2. The relative configuration of **1** was established by a NOESY experiment (*Fig. 1*), in which the correlations H–C(1)/H–C(2) and Me(14); H–C(2)/H_β–C(3), H–C(5), and Me(15); H–C(5)/Me(15) indicated that H–C(1), H–C(5), Me(15), and the MeO group at C(2) were β-, β-, β-, and α-oriented, respectively. Therefore, the structure of **1** was established as 5α-acetoxy-2α-methoxy-6-oxo-1,8-epoxygermacra-7(11),9-dien-6-oxo-8,12-olide and named myrrhanolide D.

Myrrhasin A (**2**) was isolated as yellow oil, which displayed a pseudo molecular ion peak at m/z 329.5 ($[M + Na]^+$) in the positive-ion mode ESI-MS. Its molecular formula was determined as $C_{17}H_{22}O_5$ from the $[M + H]^+$ ion peak at m/z 307.1552 (calc.

Table. *^{1}H- and ^{13}C-NMR Data* (600 and 150 MHz, resp., in $CDCl_3$) *of Compounds* **1** *and* **2**. δ in ppm, J in Hz. Atom numbering as indicated in the *Formulae.*

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	5.21 (br. *s*)	88.6	3.27 (*d*, *J* = 11.4)	67.9
2	3.76 (br. *s*)	76.3	1.87–1.92 (*m*, H_α), 1.43–1.47 (*m*, H_β)	26.3
3	1.62 (br. *s*)	31.4	1.50–1.55 (*m*, H_α),	29.0
	1.62 (br. *s*)		1.75–1.80 (*m*, H_β)	
4	2.40 (br. *s*)	32.6	2.40–2.45 (*m*)	33.0
5	5.10 (*d*, *J* = 9.0)	78.5	5.88 (br. *s*)	78.2
6		199.6		192.0
7		152.3		121.2
8		115.5		156.4
9	5.41(*s*)	121.7	3.61 (*d*, *J* = 15.0, H_a), 2.98 (*d*, *J* = 15.0, H_b)	38.3
10		146.5		58.9
11		134.1		123.6
12		169.3	7.11 (*s*)	138.7
Me(13)	1.87 (*s*)	9.9	2.15 (*s*)	10.4
Me(14)	1.86 (*s*)	13.1	1.11 (*s*)	18.0
Me(15)	1.16 (*d*, *J* = 6.0)	16.1	1.02 (*d*, *J* = 6.6)	15.4
AcO	2.08 (*s*)	20.1, 170.2	2.19 (*s*)	20.6, 171.2
MeO	3.41 (*s*)	57.4		

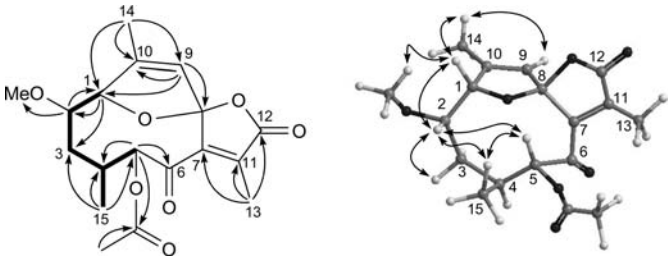


Fig. 1. *Key ^{1}H,^{1}H-COSY* (—), *HMBC* (H → C), *and NOESY* (H ↔ H) *correlations of* **1**

307.1545) in the HR-ESI-MS. In the ^{13}C-NMR spectrum (*Table*), we observed 17 signals. They were attributed to four Me, three sp^3 CH_2, and three sp^3 CH groups, one quaternary C-atom, two C=C bonds, and two C=O groups, with the aid of HMQC spectrum. The signals at δ(C) 121.2, 156.4, 123.6, and 138.7 were characteristic of furan ring C-atoms (C(7), C(8), C(11), and C(12)) in the skeleton of furanosesquterpenoids [15][16]. Consistent with the ^{13}C-NMR data, the ^{1}H-NMR spectrum revealed the presence of four Me groups and one olefinic H-atom (δ(H) 7.11) in the furan ring. Furthermore, the signals at δ(C) 20.6 and 171.2, as well as at δ(H) 2.19, indicated the presence of an AcO substitutent. The ^{1}H,^{1}H-COSY spectrum led to the identification of two partial structures: CH(1)–CH_2(2)–CH_2(3)–CH(4)–Me(15) and CH(4)–CH(5) (*Fig. 2*). Connections of these fragments to give a furanogermacrane skeleton were

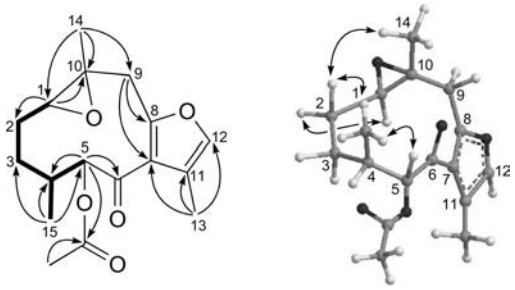


Fig. 2. *Key* $^1H,^1H$*-COSY* (—), *HMBC* (H → C), *and NOESY* (H ↔ H) *correlations of* **2**

revealed by HMBC correlations Me(14)/C(1), C(9), and C(10); H–C(9)/C(7) and C(8); Me(15)/C(3), C(4), and C(5); and H–C(5)/C(6), and C-atom signal at δ(C) 171.2 (*Fig. 2*). This established that the planar structure of **2** (*Fig. 2*) was very similar to that of **3**, except for the absence of MeO at C(2), and the replacement of C(1)=C(10) bond by 1,10-epoxy group [17]. The relative configuration of **2** was determined by NOESY correlation analysis. Specifically, correlations H_β–C(2)/Me(14) and Me(15), H–C(5)/Me(15); and H–C(1)/H_α–C(2) indicated that H–C(1), H–C(5), and Me(15) were α-, β-, and β-oriented, respectively. Accordingly, the structure of **2** was elucidated as 5α-acetoxy-1,10-epoxyfuranogermacra-7,11-dien-6-one, and named myrrhasin A.

The eight known sesquiterpenoids from the resin of *C. opobalsamum* were identified as 5-acetoxy-2-methoxyfuranogermacr-1(10)-en-6-one (**3**) [17], (1(10)*E*,2*R*,4*R*)-2-methoxy-8,12-epoxygermacra-1(10),7,11-tetraen-6-one (**4**), [15][16], (1*E*)-3-methoxy-8,12-epoxygermacra-1,7,10,11-tetraen-6-one (**5**) [15], 7α,11-dihydroxycadin-10(14)-ene (**6**) [18], myrrhone (**7**) [19], 4α,10α-dihydroxy-1α,5αH-guaia-6-ene (**8**) [20], stigmasterol (**9**) [6], and cycloartan-24-ene-1α,2α,3β-triol (**10**) [7] by comparison of their NMR data with those reported in the literature.

Compounds **1**, **2**, **6**, and **8** were isolated by our group for the first time, and were evaluated for their cytotoxic activities against PC3 and DU145 human prostate tumor cell lines using the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay [21]. However, all of the tested compounds were inactive against both cancer cell lines ($IC_{50} > 50$ μM).

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh, 10–40 μm; *Qingdao Haiyang Chemical Co. Ltd.*, China). TLC: Silica gel GF_{254} plates; visualization by heating the plates and spraying with 10% H_2SO_4/EtOH. Optical rotations: *GYROMAT-HP* polarimeter. 1D- and 2D-NMR spectra: *Bruker Avance 600* spectrometer at 600 (^{1}H) and 150 (^{13}C) MHz; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI-MS: *API 4000* mass spectrometer; in *m*/*z*. HR-ESI-MS: *LTQ-Orbitrap XL* mass spectrometer; in *m*/*z*.

Plant Material. The resin of *C. opobalsamum* was purchased in August 2010 from Jinan Jianlian Drug Store, P. R. China. It was imported from India and identified by Prof. *Lan Xiang*, School of Pharmaceutical Sciences, Shandong University, P. R. China. A voucher specimen (No. 20100817CO) has been deposited with the Department of Pharmacognosy, School of Pharmaceutical Sciences, Shandong University, P. R. China.

Extraction and Isolation. The powdered resins of *C. opobalsamum* (3.8 kg) were extracted by refluxing for 3 h with AcOEt (3×7 l). After removal of AcOEt under reduced pressure, the extract (473 g) was suspended into H_2O, and partitioned with Petroleum (PE) and CH_2Cl_2. The CH_2Cl_2 extract (297 g) was subjected to CC (SiO_2; PE/AcOEt $100:0 \rightarrow 0:100$) to give nine fractions, *Fr. A – Fr. I*. *Fr. B* (65 g) was separated by CC (SiO_2; PE/AcOEt $100:0 \rightarrow 90:10$) to afford three subfractions, *Fr. B1 – Fr. B3*. *Fr. B1* was purified by CC (SiO_2; PE/AcOEt 50:1) to give *Frs. B1a – B1c*. Compound **3** (42 mg) was crystallized from *Fr. B1a* and purified by recrystallization (PE/Me_2O). *Fr. B1b* was subjected to HPLC (*ODS*; MeOH/H_2O 85:15) to give **5** (7 mg) and **7** (7 mg). Compounds **4** (5 mg) and **9** (6 mg) were purified from *Fr. B1c* by HPLC (*ODS*; MeOH/H_2O 80:20). *Fr. B3* was separated by CC (*Sephadex LH-20*; MeOH/$CHCl_3$ 1:1) to give *Frs. B3a* and *B3b*. *Fr. B3a* was submitted to CC (SiO_2; PE/AcOEt 40:1) and further purified by HPLC (*ODS*; MeOH/H_2O 80:20) to afford **2** (10 mg). *Fr. C* (16.1 g) was subjected to CC (SiO_2; PE/AcOEt $100:0 \rightarrow 85:15$) to give two subfractions, *Fr. C1* and *Fr. C2*. Compound **1** was obtained from *Fr. C1* by HPLC (*ODS*; MeOH/H_2O 75:25. *Fr. E* (19.7 g) was separated by CC (SiO_2; (PE/AcOEt $100:0 \rightarrow 75:25$) to provide two subfractions, *Frs. E1* and *Fr. E2*. Compound **6** (9 mg) and **8** (7 mg) were isolated from *Fr. E2* by HPLC (*ODS*; MeOH/H_2O 73:27). *Fr. F* (27.2 g) was subjected to CC (SiO_2; PE/AcOEt $100:0 \rightarrow 60:40$) to give three subfractions, *Fr. F1 – Fr. F3*. Compound **10** (14 mg) was purified from *Fr. F2* by HPLC (*ODS*; MeOH/H_2O 68:32).

Myrrhanolide D (*=(5S,6S,8S,9R,11aS)-8-Methoxy-3,6,10-trimethyl-2,4-dioxo-4,5,6,7,8,9-hexahydro-2H-9,11a-epoxycyclodeca[b]furan-5-yl Acetate*; **1**). Yellow oil. $[\alpha]_D^{20} = +46.9$ ($c = 0.15$, $CHCl_3$). ^{1}H- and ^{13}C- NMR: see the *Table*. ESI-MS: 373.2 (20, $[M+Na]^+$), 368.3 (100, $[M+NH_4]^+$), 351.4 (60, $[M+H]^+$). HR-ESI-MS: 351.1454 ($[M+H]^+$, $C_{18}H_{23}O_5^+$; calc. 351.1444).

Myrrhasin A (*=(4S,5S,10aS)-4,7,10a-Trimethyl-6-oxo-1a,2,3,4,5,6,10,10a-octahydrooxireno[8,9]cyclodeca[1,2-b]furan-5-yl Acetate*; **2**). Yellow oil. $[\alpha]_D^{20} = -38.3$ ($c = 0.26$, $CHCl_3$). ^{1}H- and ^{13}C- NMR: see the *Table*. ESI-MS: 329.5 (50, $[M+Na]^+$), 307.5 (100, $[M+H]^+$). HR-ESI-MS: 307.1552 ($[M+H]^+$, $C_{17}H_{23}O_5^+$; calc. 307.1545).

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