Two New Sesquiterpenes from Myrrh

by **Nan Zhao**[a])[b]), **Guo-Chun Yang**[a])[b]), **Da-Hong Li**[a])[b]), **Xin-Yu Li**[a]), **Zhan-Lin Li**[a])[b]), **Jiao Bai**[a])[b]), **Xiao-Qiu Liu***[a]), and **Hui-Ming Hua***[a])[b])

[a]) School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Wenhua Road 103, Shenyang 110016, P. R. China (phone: +86-24-23986469 (*X.-Q. L.*), +86-24-23986465 (*H.-M. H.*); fax: +86-24-23986469 (*X.-Q. L.*), +86-24-23986465 (*H.-M. H.*); e-mail: liuxiaoqiu3388@163.com (*X.-Q. L.*), huimhua@163.com (*H.-M. H.*))

[b]) Key Laboratory of Structure-Based Drug Design and Discovery, Shenyang Pharmaceutical University, Ministry of Education, Shenyang 110016, P. R. China

Two new sesquiterpenes, named 9-methoxymyrrhone (**1**) and *rel*-(1*S*,2*S*,3*R*,4*S*)-1,2-epoxy-3-methoxyfuranogermacr-10(15)-en-6-one (**2**), together with nine known compounds (**3**–**11**), were isolated from the resin of *Commiphora opobalsamum*. The structures were established by HR-ESI-MS, 1D-, and 2D-NMR spectroscopic analyses.

Introduction. – Myrrh, one of the oldest herbal medicines and flavoring agents, is the oleo-gum-resin obtained from trees of *Commiphora* species of the Burseraceae family. As a traditional Chinese medicine, its main effect is to relieve painful swelling and pains due to blood stagnation, traumatic injury, or other kinds of wounds [1]. It is known as a rich source of secondary metabolites, such as sesquiterpenes, triterpenes, steroids, and lignans [2–8]. The contemporary pharmacology research showed that myrrh and its constituents possess various bioactivities, such as cytotoxic, antifungal, antibacterial, antalgic, antioxidant, and anti-inflammatory properties [9–11].

As part of our ongoing search for chemical constituents of myrrh, two new sesquiterpenes, 9-methoxymyrrhone (**1**) and *rel*-(1*S*,2*S*,3*R*,4*S*)-1,2-epoxy-3-methoxyfuranogermacr-10(15)-en-6-one (**2**), along with the nine known compounds (**3**–**11**; *Fig. 1*), were obtained from the CH_2Cl_2 extract of the resin of *Commiphora opobalsamum*. Herein, the isolation and structure determination of **1** and **2** are reported.

Results and Discussion. – Compound **1** was obtained as colorless needles (CH_2Cl_2). The molecular formula was determined as $C_{16}H_{18}O_3$ by the *quasi*-molecular-ion peak (*m*/*z* 281.1139 ($[M+Na]^+$; calc. 281.1154)) in the positive-ion mode HR-ESI-MS, and implied eight degrees of unsaturation. ^{1}H-NMR (600 MHz, $CDCl_3$) spectroscopic analysis revealed the presence of a sesquiterpene, endowed with a pentasubstituted benzofuran unit (δ(H) 7.38 (*q*, *J* = 1.2, 1 H)), three Me groups (1.17 (*d*, *J* = 6.0, 3 H), 2.41 (*d*, *J* = 1.2, 3 H), 2.61 (br. *s*, 3 H)), and one MeO group (3.99 (*s*, 3 H)). The ^{13}C-NMR (150 MHz, $CDCl_3$) spectrum indicated 16 C-atoms, including four Me (three Me (δ(C) 11.2, 13.8, 21.5) and one MeO (δ(C) 60.8)), one C=O C-atom (δ(C) 200.1), eight aromatic C-atoms (δ(C) 117.2, 125.6, 127.5, 128.9, 135.4, 142.0, 142.3, 150.0), and

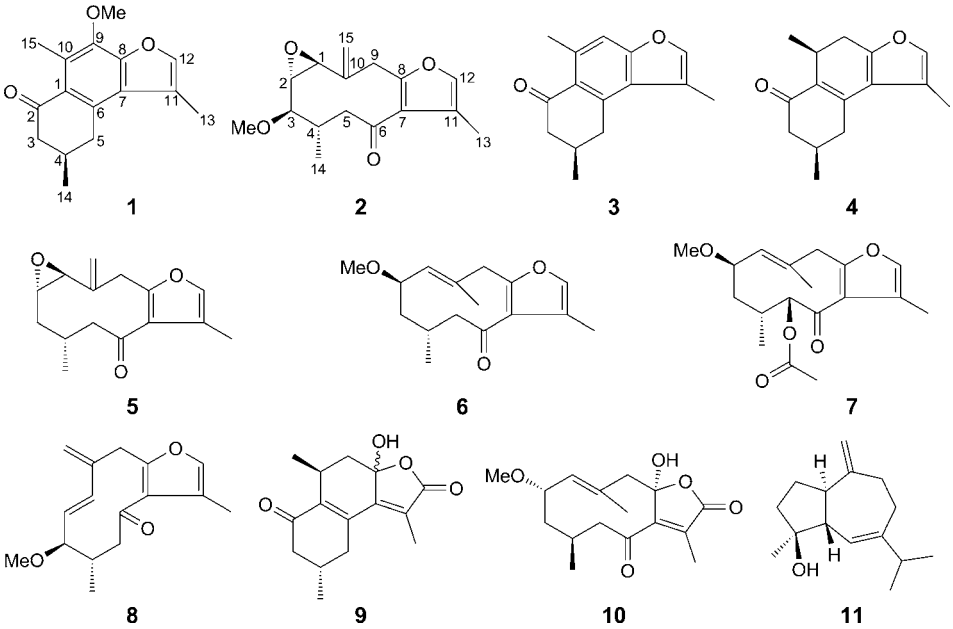

Fig. 1. *Structures of compounds* **1**–**11**

three aliphatic C-atoms (δ(C) 30.0, 35.3, 48.9). These data, together with eight degrees of molecular unsaturation, indicated that compound **1** was a close analogue of myrrhone (**3**) [12], a well-known compound isolated from myrrh, with the difference of the presence of a MeO group and the lack of one aromatic H-atom signal in **1**. The location of the MeO substituent was elaborated by an HMBC between the MeO signal at δ(H) 3.99 and C(9) (δ(C) 142.3) (*Fig. 2*). The completely assigned ^{1}H- and ^{13}C-NMR signals are shown in the *Table*. Therefore, compound **1** was determined as 9-methoxymyrrhone.

Compound **2** was isolated as a colorless oil, and the *quasi*-molecular-ion peak at *m*/*z* 299.1248 ($[M+\mathrm{Na}]^+$) in the HR-ESI-MS established the molecular formula $C_{16}H_{20}O_4$, indicating seven degrees of unsaturation. ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of **2** exhibited signals due to one terminal C=C bond (δ(H) 5.16 (br. *s*, 1 H), 4.95 (br. *s*, 1 H)), two Me groups (1.09 (*d*, $J = 6.6$, 3 H), and 2.05 (*d*, $J = 1.0$, 3 H)), one MeO group (3.52 (*s*, 3 H)), and one downfield olefin H-atom at δ(H) 7.06 (*q*, $J = 1.0$, 1 H). The

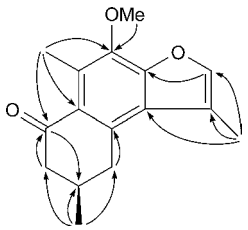

Fig. 2. *Key HMBCs* (H → C) *of* **1**

Table. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2**. δ in ppm, J in Hz

Position	**1**[a])		**2**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)
1		127.5	2.91 (br. *s*)	56.0
2		200.1	2.36 (*dd*, *J* = 8.9, 2.1)	65.8
3	2.71–2.73 (*m*), 2.35–2.38 (*m*)	48.9	2.46 (*d*, *J* = 8.9)	86.1
4	2.33	30.0	2.18–2.24 (*m*)	34.6
5	3.48–3.51 (*m*), 2.82 (*dd*, *J* = 16.2, 10.2)	35.3	2.70–2.76 (*m*), 2.30–2.34 (*m*)	52.1
6		135.4		201.0
7		125.6		129.0
8		150.0		153.9
9		142.3	3.45 (br. *d*, *J* = 14.5), 2.66 (*d*, *J* = 14.5)	37.5
10		128.9		141.0
11		117.2		117.8
12	7.38 (*q*, *J* = 1.2)	142.0	7.06 (*q*, *J* = 1.0)	138.7
Me(13)	2.41 (*d*, *J* = 1.2)	11.2	2.05 (*d*, *J* = 1.0)	9.6
Me(14)	1.17 (*d*, *J* = 6.0)	21.5	1.09 (*d*, *J* = 6.6)	18.3
Me(15)	2.61 (br. *s*)	13.8	5.16 (br. *s*), 4.95 (br. *s*)	112.8
MeO	3.99 (*s*)	60.8	3.52 (*s*)	59.5

[a]) 600 and 150 MHz in $CDCl_3$. [b]) 400 and 100 MHz in $CDCl_3$.

^{13}C-NMR spectrum displayed 16 C-atom signals, which were assigned by a DEPT experiment as two Me, one MeO group, two aliphatic and one olefinic CH_2 groups, four aliphatic and one olefinic CH groups, four olefinic C_q-atoms, and one C=O C-atom, suggesting that **2** was a furanosesquiterpene. The NMR data of **2** exhibited close similarity to those of *rel*-(1*S*,2*S*,4*R*)-1,2-epoxy-furanogermacr-10(15)-en-6-one (**5**) [13], except for an extra MeO group, which was located at C(3), as confirmed by the HMBC correlation MeO/C(3). The relative configuration of **2** was determined by the similarity of the coupling constants compared to those of **5**. This contention was confirmed by the NOESY correlations between H–C(1) (δ(H) 2.91)/H–C(3) (δ(H) 2.46), H–C(2) (δ(H) 2.36)/MeO (δ(H) 3.52), H–C(3) (δ(H) 2.46)/Me(14) (δ(H) 1.09), respectively (*Fig. 3*). Therefore, the structure of **2** was elucidated as *rel*-(1*S*,2*S*,3*R*,4*S*)-1,2-epoxy-3-methoxyfuranogermacr-10(15)-en-6-one, its absolute configuration remaining to be determined.

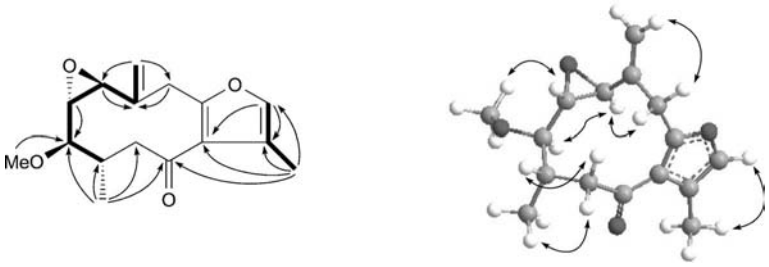

Fig. 3. *Key ^{1}H,^{1}H-COSY* (▬), *HMBC* (H → C), *and NOESY* (H ↔ H) *correlations of* **2**

The known compounds were identified as myrrhone (**3**) [12], agarsenone (**4**) [14], *rel*-(1*S*,2*S*,4*R*)-1,2-epoxyfuranogermacr-10(15)-en-6-one (**5**) [13], (1(10)*E*,2*R**,4*R**)-2-methoxy-8,12-epoxygermacra-1(10),7,11-trien-6-one (**6**) [15], (1(10)*E*,2*R**,5*R**)-2-methoxy-5-acetoxyfuranogermacr-1(10)-en-6-one (**7**) [16], *rel*-(1*E*,3*R*,4*S*)-methoxy-4-furanogermacra-1,10(15)-dien-6-one (**8**) [13], myrrhanolides B and C (**9**) [17], 2α-methoxy-8α-hydroxy-6-oxogermacra-1(10),7(11)-dien-12,8-olide (**10**) [18], and alismol (**11**) [19] by various spectroscopic analyses and comparison with the data reported in the literature.

We are grateful to Mr. *Y. Sha* and Mrs. *W. Li* for measurements of the NMR data and Prof. *Q.-S. Sun* for material identification. Financial support of the *National Natural Science Foundation of China* (Grant No. 81172958) and *Training Programs of Innovation and Entrepreneurship for Undergraduates* are gratefully acknowledged.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 100–200 and 200–300 mesh; *Qingdao Haiyang Chemical Co., Ltd.*, P. R. China), *Sephadex LH-20* (*Amersham Co. Ltd.*) and *ODS* (C_{18} reversed-phase (RP) SiO_2; 50 μm; *GE Healthcare*). Optical rotations: *JASCO P-2000* automatic polarimeter. ^{1}H- and ^{13}C-NMR spectra: *Bruker ARX-400* and *AV-600* NMR spectrometers; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *Autospec-VltimaE TOF* mass spectrometer, in *m*/*z*.

Plant Material. The resin of *Commiphora opobalsamum* was purchased from Beijing Tongrentang, Shenyang, P. R. China, in March 2007, and identified by Prof. *Q.-S. Sun* (School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, P. R. China). A voucher specimen (MY20070320) was deposited with the Department of Natural Products Chemistry, Shenyang Pharmaceutical University, Shenyang, P. R. China.

Extraction and Isolation. The powdered myrrh (900 g) was extracted three times (3 × 1.5 h) with CH_2Cl_2 under reflux. The crude extract (400 g) was subjected to CC (SiO_2; PE/AcOEt 100:0, 100:5, 100:10, 100:20, 100:50) to yield five fractions (*Frs. A–E*). *Fr. B* (67.0 g) was separated by CC (SiO_2; PE/AcOEt 100:0 → 100:5) to give five subfractions, *Frs. B1–B5*, then *Fr. B4* (12 g) was further purified by recrystallization to yield **7** (500 mg). *Fr. B2* (6 g) was submitted to CC (*ODS*; MeOH/H_2O 40:60) and then separated by semi-prep. HPLC (MeOH/H_2O 73:27) to give **6** (100 mg), **3** (30 mg), **1** (37 mg), **4** (23 mg), and **8** (8 mg). *Fr. B3* (1 g) was further purified by semi-prep. HPLC (MeOH/H_2O 73:27) to obtain **5** (10 mg) and **11** (8 mg). *Fr. D* (34.0 g) was separated by CC (*ODS*; MeOH/H_2O 40:60 → 60:40) and further by HPLC (MeOH/H_2O 78:22) to yield **9** (15 mg), **10** (10 mg), and **2** (6 mg).

9-Methoxymyrrhone (=*(8*R*)-8,9-Dihydro-4-methoxy-1,5,8-trimethylnaphtho[2,1-*b*]furan-6(7*H*)-one*; **1**). Colorless needles (CH_2Cl_2). $[\alpha]_D^{20} = -15.2$ (*c* = 2.52, CH_2Cl_2). ^{1}H- and ^{13}C-NMR ($CDCl_3$): see the *Table*. HR-ESI-MS: 281.1139 ($[M+Na]^+$, $C_{16}H_{18}NaO_3^+$; calc. 281.1154).

rel-*(1*S*,2*S*,3*R*,4*S*)-1,2-Epoxy-3-methoxyfuranogermacr-10(15)-en-6-one* (=rel-(*1a*S*,2*R*,3*S*,10a*S*)-1a,3,4,9,10,10a-Hexahydro-2-methoxy-3,6-dimethyl-10-methylideneoxireno[7,8]cyclodeca[1,2-*b*]furan-5(2*H*)-one*; **2**). Colorless oil. $[\alpha]_D^{20} = +11.7$ (*c* = 0.29, CH_2Cl_2). ^{1}H- and ^{13}C-NMR ($CDCl_3$): see the *Table.* HR-ESI-MS: 299.1248 ($[M+Na]^+$, $C_{16}H_{20}NaO_4^+$; calc. 299.1254).

REFERENCES

[1] P.-G. Xiao, in 'Modern Chinese Material Medica', Chemical Industry Press, Beijing, 2001, Vol. 3, p. 851.
[2] L. O. Hanuš, T. Řezanka, V. M. Dembitsky, A. Moussaieff, *Biomed. Papers* **2005**, *149*, 3.
[3] T. Shen, H.-X. Lou, *Nat. Prod. Res. Dev.* **2008**, *20*, 360.
[4] E. S. H. El Ashry, N. Rashed, O. M. Salama, A. Saleh, *Pharmazie* **2003**, *58*, 163.

[5] T. Shen, H.-Q. Yuan, W.-Z. Wan, X.-L. Wang, X.-N. Wang, M. Ji, H.-X. Lou, *J. Nat. Prod*. **2008**, *71*, 81.
[6] G. J. Provan, P. G. Waterman, *Phytochemistry* **1988**, *27*, 3841.
[7] L. O. A. Manguro, I. Ugi, P. Lemmen, *Chem. Pharm. Bull*. **2003**, *51*, 483.
[8] A. Dekebo, M. Lang, K. Polborn, E. Dagne, W. Steglich, *J. Nat. Prod*. **2002**, *65*, 1252.
[9] T. Shen, G.-H. Li, X.-N. Wang, H.-X. Lou, *J. Ethnopharmacol.* **2012**, *142*, 319.
[10] X.-L. Wang, F. Kong, T. Shen, C. Y. F. Yong, H.-X. Lou, H.-Q. Yuan, *Acta Pharm. Sin*. **2011**, *32*, 338.
[11] M. Duwiejua, I. J. Zeitlin, P. G. Waterman, J. Chapman, G. J. Mhango, G. J. Provan, *Planta Med*. **1993**, *59*, 12.
[12] N. Zhu, S. Sheng, S. Sang, R. T. Rosen, C.-T. Ho, *Flavour Fragrance J.* **2003**, *18*, 282.
[13] N. Zhun, H. Kikuzaki, S. Sheng, S. Sang, M. M. Rafi, M. Wang, N. Nakatani, R. S. DiPaola, R. T. Rosen, C.-T. Ho, *J. Nat. Prod.* **2001**, *64*, 1460.
[14] S. Santoro, S. Superchi, F. Messina, E. Santoro, O. Rosati, C. Santi, M. C. Marcotullio, *J. Nat. Prod.* **2013**, *76*, 1254.
[15] A. Dekebo, E. Dagne, L. K. Hansen, O. R. Gautun, A. J. Aasen, *Tetrahedron Lett.* **2000**, *41*, 9875.
[16] C. H. Brieskorn, P. Noble, *Tetrahedron Lett.* **1980**, *21*, 1511.
[17] T. Shen, W.-Z. Wan, X.-N. Wang, H.-Q. Yuan, M. Ji, H.-X. Lou, *Helv. Chim. Acta* **2009**, *92*, 645.
[18] J.-L. Yang, Y.-P. Shi, *Phytochemistry* **2012**, *76*, 124.
[19] G.-P. Peng; G. Tian, X.-F. Huang, F.-C. Lou, *Phytochemistry* **2003**, *63*, 877.

Received April 19, 2015