Sesquiterpenoids from the Resinous Exudates of *Commiphora opobalsamum* (Burseraceae)

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Phytochemical investigation of the exudates of *Commiphora opobalsamum* led to the isolation of three new sesquiterpenoids, 2α-methoxy-6-oxogermacra-1(10),7(11)-dien-8,12-olide (**1**), 5β-10α-hydroxy-2α-methoxy-6-oxoguaia-7(11),8-dien-8,12-olide (**2**), and furanocadina-1(10),6,8-triene-4-ol (**3**), together with six known compounds. Their structures were elucidated on the basis of spectroscopic methods.

Introduction. – The genus *Commiphora*, belonging to the family Burseraceae, comprises over 150 species, and mainly occurs in Eastern Africa, Arabia and India [1]. The resinous exudates of these plant species possess diverse biological activities, such as cytotoxic, anaesthetic, anti-inflammatory, and antimicrobial effects, and have been used for a long time in the traditional medicines of India, China, Rome, Greece, and Babylon [2–10]. Previous phytochemical investigations of this genus resulted in the isolation of a series of terpenoids, steroids, flavonoids, and sugars [11][12].

In our continuous search for bioactive constituents from Chinese traditional medicinal plants [3], we herein report the three new sesquiterpenes **1**–**3**, along with six known compounds **4**–**9** from the resinous exudates of *C. opobalsamum*. Furthermore, the cytotoxic and antifungal activities of compounds **1**–**8** were evaluated against two human tumor cell lines and *Candida albicans*, respectively.

Results and Discussion. – The exudates of *C. opobalsamum* were extracted with petroleum ether (PE), and then purified by repeated column chromatography over silica gel and preparative TLC to afford three new sesquiterpenoids **1**–**3**, together with the known compounds **4**–**9**.

Compound **1** was isolated as colorless crystals, which showed a pseudomolecular ion at *m*/*z* 301.6 $[M+Na]^+$ in the positive-ion ESI-MS. Its molecular formula was determined to be $C_{16}H_{22}O_4$ by the M^+ peak at *m*/*z* 278.1527 (calc. 278.1518) in the HR-EI-MS. The ^{1}H-NMR spectrum showed the presence of two Me *singlet*s (δ(H) 1.75 and

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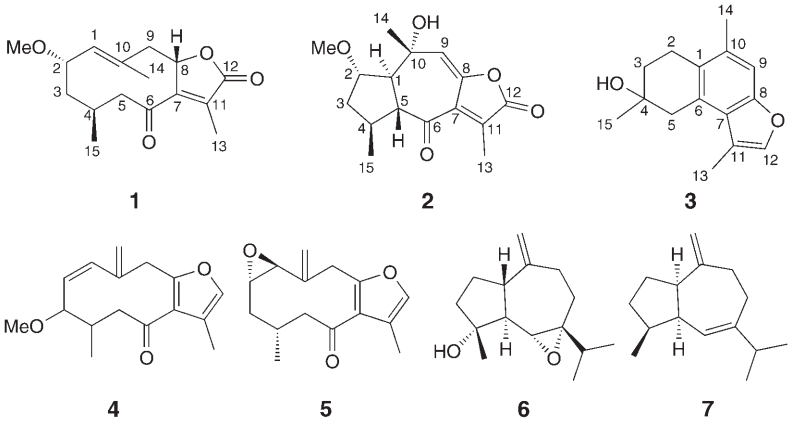

3.07), two Me *doublet*s (δ(H) 1.18 (*d*, $J = 7.2$ Hz) and 2.01 (*d*, $J = 1.8$ Hz)), two oxymethine H-atoms (δ(H) 4.04 and 5.35–5.37), and one olefinic H-atom (δ(H) 5.20 (*d*, $J = 7.6$ Hz)). The ^{13}C-NMR spectrum resolved 16 C-atom signals, which were determined by chemical shifts and the HMQC spectrum as four Me groups (one MeO group at δ(C) 56.0), three sp^3 CH_2, and three sp^3 CH groups (two oxygenated at δ(C) 75.3 and 80.9), two CO groups (δ(C) 197.9 and 172.8), and two C=C bonds (*Table*). The presence of an α,β-unsaturated α-methyl-γ-lactone moiety was confirmed by the C-atom signals at δ(C) 172.8 (C(12)), 129.6 (C(11)), 10.3 (C(13)), 158.7 (C(7)), and 80.9 (C(8)). The ^{1}H,^{1}H-COSY spectrum led to the identification of two partial structures: CH(1)–CH(2)–CH_2(3)–CH(4)–Me(15) and CH(8)–CH_2(9) (*Fig. 1*). Connection of these fragments to give a germacrane type lactone was rendered possible by the HMBC correlations of Me(14)/C(1), C(9), and C(10); H–C(5)/C(4) and C(6); Me(13)/C(6), C(7), C(11), and C(12); and H–C(8)/C(7) (*Fig. 1*). The position of the MeO group at C(2) was deduced from the cross-peak of H–C(2)/δ(C) 56.0 (MeO).

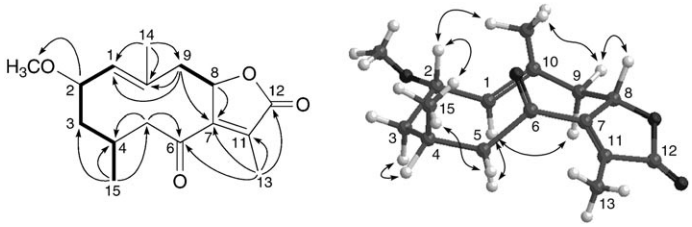

Fig. 1. *Key* ^{1}*H,*^{1}*H-COSY* (—), *HMBC* (H → C), *and NOESY* (H ↔ H) *correlations of* **1**

The relative configuration of **1** was established by a NOESY experiment (*Fig. 1*), in which the correlations of H–C(2)/Me(14) and Me(15); H_β–C(5)/Me(15); H_β–C(9)/H–C(8) and Me(14); H_α–C(5)/H–C(1) and H_α–C(9); and H_α–C(3)/H–C(4) indicated that H–C(8), Me(15), and the MeO group at C(2) were β-, β-, and α-

Table. *^{1}H- and ^{13}C-NMR Data of Compounds* **1**–**3**. At 600/150 MHz, resp.; δ in ppm, J in Hz.

	1[a])		**2**[a])		**2**[b])		**3**[a])	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1) or C(1)	5.20 (*d*, *J* = 7.6)	133.6	2.31–2.38 (*m*)	57.1	2.74 (br. *d*, *J* = 6.3)	61.7		127.1
H–C(2) or CH_2(2)	4.04 (*dt*, *J* = 2.3, 7.4)	75.3	3.99–4.01 (*m*)	82.1	4.19 (br. *d*, *J* = 5.2)	83.7	2.72–2.77 (*m*), 2.83–2.89 (*m*)	24.1
CH_2(3)	1.88–1.93 (*m*, H_α), 1.77–1.79 (*m*, H_β)	35.7	1.99–2.03 (*m*, H_α), 1.41–1.46 (*m*, H_β)	38.9	2.12 (*dd*, *J* = 5.7, 13.3, H_α), 1.26–1.31 (*m*, H_β)	41.5	1.83–1.88 (*m*), 1.95–2.00 (*m*)	35.3
H–C(4) or C(4)	2.28–2.32 (*m*)	25.0	2.83–2.85 (*m*)	31.3	2.95–3.00 (*m*)	34.1		68.3
CH_2(5) or H–C(5)	2.80–2.83 (*m*, H_α), 2.53 (*dd*, *J* = 6.3, 17.5, H_β)	47.2	2.31–2.38 (*m*)	60.2	2.56 (*t*, *J* = 10.2)	63.5	3.22–3.29 (*m*)	41.0
C(6)		197.9		193.5		195.8		127.8
C(7)		158.7		144.8		145.7		124.6
H–C(8) or C(8)	5.35–5.37 (*m*)	80.9		139.1		140.7		154.0
CH_2(9) or H–C(9)	2.10 (*t*, *J* = 11.8, H_α), 2.80–2.83 (*m*, H_β)	45.8	5.97 (*s*)	121.2	6.27 (*s*)	124.5	7.13 (*s*)	110.3
C(10)		133.6		72.6		72.5		133.0
C(11)		129.6		137.9		137.5		115.7
C(12) or H–C(12)		172.8		168.9		170.2	7.26 (*s*)	140.6
Me(13)	2.01 (*d*, *J* = 1.8)	10.3	2.35 (*s*)	11.0	2.20 (*s*)	11.7	2.38 (*s*)	11.0
Me(14)	1.75 (*s*)	17.4	1.42 (*s*)	22.3	1.53 (*s*)	23.6	2.32 (*s*)	20.2
Me(15)	1.18 (*d*, *J* = 7.2)	22.8	1.09 (*d*, *J* = 6.7)	19.7	1.16 (*d*, *J* = 6.5)	19.6	1.41 (*s*)	28.4
MeO	3.07 (*s*)	56.0	3.32 (*s*)	55.5	3.33 (*s*)	56.9		

[a]) In $CDCl_3$. [b]) In (D_5)pyridine.

oriented, respectively. Therefore, the structure of **1** was assigned to be 2α-methoxy-6-oxogermacra-1(10),7(11)-dien-8,12-olide[2]), but its absolute configuration remains to be established.

Compound **2**, a white amorphous powder, exhibited a molecular ion peak M^+ at m/z 292.1310 (calc. 292.1311) in the HR-EI-MS, which is consistent with the molecular formula $C_{16}H_{20}O_5$. The ^{1}H-NMR spectrum showed the presence of three Me *singlet*s (δ(H) 1.42, 2.35, and 3.32), one Me *doublet* (δ(H) 1.09, d, J = 6.7 Hz), and one olefinic H-atom (δ(H) 5.97, s). The ^{13}C-NMR spectrum displayed signals for 16 C-atoms. By HMQC, they were determined to consist of four Me groups (one MeO group at δ(C) 55.5), one sp^3 CH_2 (δ(C) 38.9), and four sp^3 CH groups (one oxygenated at δ(C) 82.1), one oxygenated quaternary sp^3 C-atom (δ(C) 72.6), two CO groups (δ(C) 193.5 and 168.9), one trisubstituted C=C bond, and one tetrasubstituted C=C bond (*Table*). The C-atom signals at δ(C) 168.9 (C(12)), 137.9 (C(11)), 11.0 (C(13)), 144.8 (C(7)), and 139.1 (C(8)), were undoubtedly assigned to an α,β-unsaturated α-methyl-γ-lactone ring. The ^{1}H,^{1}H-COSY spectrum of **2** confirmed the presence of a five-membered ring (depicted with bold lines in *Fig. 2*). Combined with the HMBC correlations of Me(14)/C(1), C(9), and C(10); H–C(9)/C(7) and C(8); H–C(5)/C(1) and C(6); and Me(13)/C(7), C(11), and C(12) (*Fig. 2*), compound **2** was established as a guaiane type lactone.

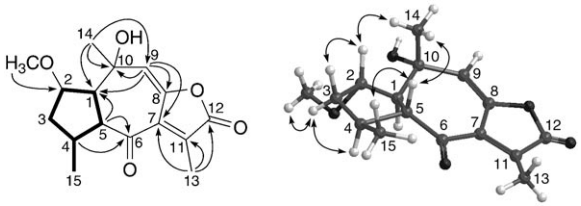

Fig. 2. *Key* ^{1}*H,*^{1}*H-COSY* (—), *HMBC* (H → C), *and NOESY* (H ↔ H) *correlations of* **2**

Due to signal overlapping of H–C(1) with H–C(5) at δ(H) 2.31–2.38 (m) in $CDCl_3$, we recorded the ^{1}H-NMR spectrum in (D_5)pyridine, which caused that the H–C(1) resonance was shifted downfield *ca.* $\Delta\delta$ 0.39 to δ(H) 2.74, while H–C(5) was shifted downfield $\Delta\delta$ 0.21 to δ(H) 2.56 (*Table*). The relative configuration of **2** was established by a NOESY experiment in (D_5)pyridine. The NOESY correlations (*Fig. 2*) of H–C(2)/H_β–C(3) and Me(14); H_α–C(3)/H–C(4) and the MeO group at C(2); and H–C(5)/Me(14) and Me(15) showed that H–C(2), H–C(5), Me(14), and Me(15) were β-oriented, whereas H–C(1), the OH group at C(10), and the MeO group at C(2) were α-oriented. Thus, the structure of **2** was deduced as 5β-10α-hydroxy-2α-methoxy-6-oxoguaia-7(11),8-dien-8,12-olide[2]).

Compound **3** was isolated as a yellow oil. The molecular formula was determined to be $C_{15}H_{18}O_2$ on the basis of the M^+ peak at m/z 230.1299 (calc. 230.1307) in the HR-EI-MS. The ^{1}H-NMR spectrum showed the presence of three Me *singlet*s (δ(H) 1.41, 2.32, and 2.38), and two downfield signals at δ(H) 7.26 (1 H, s) and 7.13 (1 H, s). The ^{13}C-NMR spectrum exhibited signals for 15 C-atoms including three Me groups (δ(C)

[2]) For systematic names, see *Exper. Part.*

11.0, 20.2, and 28.4), three CH_2 groups (δ(C) 24.1, 35.3, and 41.0), and one oxygenated quaternary C-atom (δ(C) 68.3), two trisubstituted C=C bonds, and two tetrasubstituted C=C bonds (*Table*). The ^{1}H- and ^{13}C-NMR data suggested that the structure of **3** was very similar to that of the known furanosesquiterpenoid dihydropyrocurzerenone [13], which was verified by analysis of its HMQC, ^{1}H,^{1}H-COSY, and HMBC spectra (*Fig. 3*). The only difference was that the resonance for C(4) was shifted from δ(C) 28.6 to δ(C) 68.3, indicating that the methine H-atom at C(4) in dihydropyrocurzerenone was replaced by an OH group in **3**. Therefore, the structure of **3** was established as furanocadina-1(10),6,8-triene-4-ol[2]).

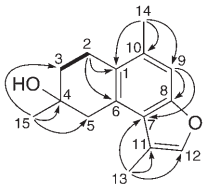

Fig. 3. *Key ^{1}H,^{1}H-COSY* (—) *and HMBC* (H → C) *correlations of* **3**

The known compounds **4**–**9** were identified as (1*E*)-3-methoxy-8,12-epoxygermacra-1,7,10,11-tetraen-6-one (**4**) [13], *rel*-(1*S*,2*S*)-epoxy-(4*R*)-furanogermacr-10(15)-en-6-one (**5**) [14], 6α,7α-epoxy-1β-guai-10(14)-en-4α-ol (**6**) [15], (1*R*,4*S*,5*R*)-guaia-6,10(14)-diene (**7**) [16], cerotic acid (**8**) [17], and β-sitosterol (**9**) [17] by comparison of their ^{1}H- and ^{13}C-NMR data with those reported in the cited references.

The antifungal activities of compounds **1**–**8** were tested against *Candida albicans*. Unfortunately, all test compounds were inactive (MIC > 64 μg/ml). The cytotoxicities of **1**–**8** were evaluated against the PC3 (human prostate tumor) and HT29 (human colorectal carcinoma) cell lines. However, they were inactive against both human tumor cell lines (IC_{50} > 50 μM).

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Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh, 10–40 μm; *Qingdao Haiyang Chemical Co. Ltd.*, China). TLC: silica gel GF_{254} plates; visualization by heating the plates sprayed with 10% H_2SO_4/EtOH. M.p.: *X-6* melting-point apparatus (*Beijing TECH Instrument Co. Ltd.*, China). Optical rotations: *Perkin-Elmer 241 MC* polarimeter. UV: *Agilent 8453E* UV/VIS Spectrometer. IR: *Nexus 470* FT-IR Spectrometer. 1D- and 2D-NMR spectra: *Bruker Avance 600* spectrometer at 600 (^{1}H) and 150 (^{13}C) MHz. HR-EI-MS spectra: *Waters GCT* mass spectrometer. ESI-MS spectra: *API 4000* mass spectrometer.

Plant Material. The exudates of *C. opobalsamum* were purchased in September 2002 from Affiliated Hospital of Shandong Traditional Chinese Medical University, Jinan, P. R. China. It was imported from India and identified by Prof. *Qi-Shi Sun*, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, P. R. China. A voucher specimen (No. 20020910CO) has been deposited at the Laboratory of Natural Products Chemistry, School of Pharmaceutical Sciences, Shandong University, P. R. China.

Extraction and Isolation. The resinous exudates of *C. opobalsamum* (3.5 kg) were extracted with petroleum ether (PE) in a *Soxhlet* apparatus for 36 h. The crude extract (290 g) was subjected to CC (SiO_2; PE/AcOEt 1:0 → 0:1) to provide nine fractions (*Fr. A–I*). *Fr. B* (25 g) was subjected to CC (SiO_2; PE/AcOEt 98:2 → 1:1) to afford nine subfractions (*Fr. B1–B9*). *Fr. B4* was separated by CC (SiO_2; PE/Et_2O 95:5 → 1:1) to give **8** (30 mg) and other four subfractions (*Fr. B4a–B4d*). **5** (15 mg) was isolated from *Fr. B4d* by preparative TLC (SiO_2; PE/AcOEt 3:2). **4** (45 mg) was obtained from *Fr. B4a* by preparative TLC (SiO_2; PE/$CHCl_3$/Et_2O 6:4:1). **7** (23 mg) was isolated from *Fr. B4c* by preparative TLC (SiO_2; PE/AcOEt 13:7). *Fr. B8* was subjected to CC (SiO_2; PE/AcOEt 1:1) to afford **9** (80 mg). *Fr. C* (21 g) was chromatographed over CC (SiO_2; PE/AcOEt 97:3 → 1:1) to give five subfractions (*Fr. C1–C5*). **3** (5 mg) was obtained from *Fr. C5* by CC (SiO_2; PE/Et_2O 97:3), and purified by prep. TLC (SiO_2; PE/$CHCl_3$/Et_2O 10:7:3). *Fr. D* was subjected to CC (silica gel; PE/Me_2O 97:3 → 1:1) to give seven subfractions (*Fr. D1–D7*). *Fr. D6* was separated by CC (SiO_2; PE/AcOEt 4:1) to afford **6** (8 mg). **1** (17 mg) was isolated from *Fr. D7* by CC (SiO_2; PE/Me_2O 9:1), and purified by recrystallization (PE/Me_2O). Compound **2** (6 mg) was obtained from *Fr. E* by CC (SiO_2; PE/Me_2O 93:7), and then purified by CC (SiO_2; PE/Et_2O/Me_2O 50:50:1).

2α-Methoxy-6-oxogermacra-1(10),7(11)-dien-8,12-olide (=*(6*S*,8*S*,11a*S*)-5,6,7,8,11,11a-Hexahydro-8-methoxy-3,6,10-trimethylcyclodeca[*b*]furan-2,4-dione*; **1**). Colorless crystals (PE/Me_2O). $[\alpha]_D^{20} = +196.2$ ($c = 0.053$, $CHCl_3$). UV (MeOH): 229.0 (4.08). IR (KBr): 2946, 2924, 2906, 2882, 1755, 1676, 1366, 1171, 1093, 1023, 753. ^{1}H- and ^{13}C-NMR: *Table.* ESI-MS: 301.6 (6, $[M + Na]^+$), 296.5 (100, $[M + NH_4]^+$), 279.5 (40, $[M + H]^+$). HR-EI-MS: 278.1527 (M^+, $C_{16}H_{22}O_4^+$; calc. 278.1518).

5β-10α-Hydroxy-2α-methoxy-6-oxoguaia-7(11),8-dien-8,12-olide (=*(4a*R*,5*S*,7*S*,7a*S*,8*S*)-4a,5,6,7,7a,8-Hexahydro-8-hydroxy-7-methoxy-3,5,8-trimethylazuleno[6,5-*b*]furan-2,4-dione*; **2**). White amorphous powder. $[\alpha]_D^{20} = -46.1$ ($c = 0.065$, $CHCl_3$). UV (MeOH): 220.0 (3.82), 304 (3.89). IR (KBr): 3392, 3025, 2960, 2871, 1765, 1731, 1643, 1377, 767. ^{1}H- and ^{13}C- NMR: *Table.* ESI-MS: 315.4 (58, $[M + Na]^+$), 310.7 (100, $[M + NH_4]^+$), 275.6 (75, $[M + H - H_2O]^+$). HR-EI-MS: 292.1310 (M^+, $C_{16}H_{20}O_5^+$; calc. 292.1311).

Furanocadina-1(10),6,8-triene-4-ol (=*6,7,8,9-Tetrahydro-1,5,8-trimethylnaphtho[2,1-*b*]furan-8-ol*; **3**). Yellow oil. $[\alpha]_D^{20} = -27.2$ ($c = 0.058$, $CHCl_3$). UV (MeOH): 215.0 (4.21), 246.0 (3.80), 253 (3.79). IR (KBr): 3428, 2951, 2924, 2866, 1615, 1572, 1538, 1453, 1101, 1029, 842, 766. ^{1}H- and ^{13}C- NMR: *Table.* HR-EI-MS: 230.1299 (M^+, $C_{15}H_{18}O_2^+$; calc. 230.1307).

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