Cycloartane-Type Triterpenoids from the Resinous Exudates of *Commiphora opobalsamum*

Tao Shen,[†] Hui-Qing Yuan,[‡] Wen-Zhu Wan,[†] Xiao-Ling Wang,[‡] Xiao-Ning Wang,[†] Mei Ji,[†] and Hong-Xiang Lou*[,†]

Department of Natural Products Chemistry, School of Pharmaceutical Sciences, and Department of Biochemistry and Molecular Biology, School of Medicine, Shandong University, 44 West Wenhua Road, Jinan 250012, People's Republic of China

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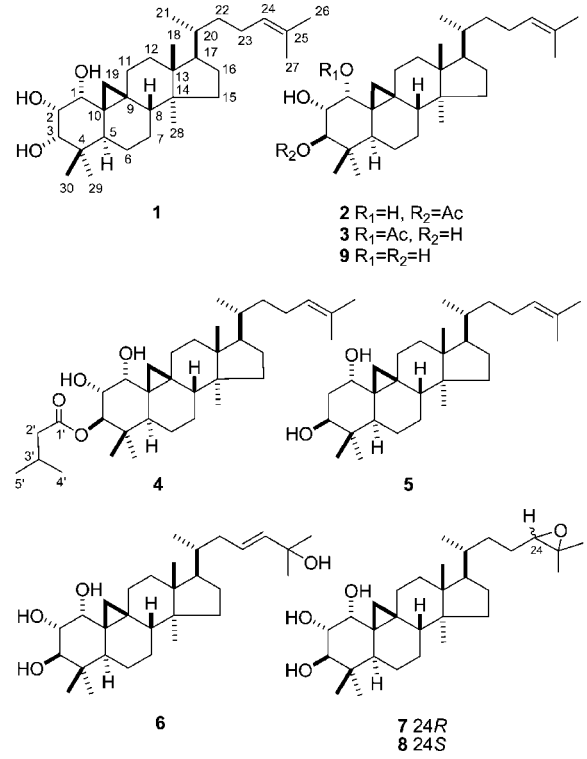
Eight new cycloartane-type triterpenoids, cycloartan-24-ene-1α,2α,3α-triol (**1**), 3*β*-acetoxycycloartan-24-ene-1α,2α-diol (**2**), 1α-acetoxycycloartan-24-ene-2α,3*β*-diol (**3**), 3*β*-isovaleroyloxycycloartan-24-ene-1α,2α-diol (**4**), cycloartan-24-ene-1α,3*β*-diol (**5**), cycloartan-23*E*-ene-1α,2α,3*β*,25-tetrol (**6**), and an epimeric mixture of 24*R*,25-epoxycycloartane-1α,2α,3*β*-triol (**7**) and 24*S*,25-epoxycycloartane-1α,2α,3*β*-triol (**8**), together with one known compound, cycloartan-24-ene-1α,2α,3*β*-triol (**9**), were isolated from the resinous exudates of *Commiphora opobalsamum*. Their structures were established on the basis of mass spectrometry and multidimensional NMR spectroscopy. The cytotoxicity of compounds **1**–**9** was evaluated against the PC3 and DU145 human prostate tumor cell lines. All of the compounds except **1** and **5** exhibited moderate cytotoxicity against PC3 or DU145 cells with IC_{50} values ranging from 10.1 to 37.2 μM.

The *Commiphora* genus in the family Burseraceae comprises over 150 species, which are mainly distributed throughout Eastern Africa, Arabia, and India.[1] The resinous exudates of these plant species have been used for a long time in the traditional medicines of India, China, Rome, Greece, and Babylon. The species of *Commiphora myrrha*,[2,3] *C. kua*,[4,5] *C. mukul*,[6–8] *C. merkeri*,[9] *C. wightii*,[10,11] *C. molmol*,[12–14] *C. pyracanthoides*,[15–17] *C. erlangeriana*,[18] *C. dalzielii*,[19] *C. confusa*,[20] and *C. sphaerocarpa*,[21,22] have been phytochemically investigated previously, and a series of metabolites including terpenoids, steroids, flavonoids, lignans, carbohydrates, and long chain aliphatic alcohol derivatives have been reported.[23,24] Secondary metabolites and crude extracts of the *Commiphora* species exhibited diverse biological activities, such as cytotoxic,[25–27] anesthetic,[28] anti-inflammatory,[29–31] and antimicrobial effects,[32,33] and were also used in dentistry for endodontic therapy and temporary fillings.[34]

In our continuous search for cytotoxic constituents against human prostate cancer from traditional Chinese medicine,[27,35] a cycloartane-type triterpenoid and an aliphatic alcohol glycoside with inhibitory effect on the expression of the androgen receptor (AR) in LNCaP cells were obtained from *C. opobalsamum*.[27] Here, we have reinvestigated the resinous exudates of *C. opobalsamum*, resulting in the discovery of eight new cycloartane-type triterpenoids (**1**–**8**), together with one known compound. In addition, all isolates were tested for their cytotoxic activities against the PC3 and DU145 human prostate tumor cell lines.



Results and Discussion

The resinous exudates of *C. opobalsamum* were extracted with petroleum ether (PE) and then subjected to Si gel column chromatography (CC) eluted with a gradient of PE−EtOAc with increasing amounts of EtOAc to provide nine fractions (A−I). Fraction F exhibited cytotoxicity against the PC3 human prostate tumor cell line with an IC_{50} value of 22.5 μg/mL. Further purification of fraction F by Si gel CC and preparative TLC resulted in the isolation of eight new cycloartane-type triterpenoids (**1**–**8**), together with one known compound (**9**).

Compound **1** was obtained as white needles. The positive ESIMS showed a pseudomolecular ion peak at *m/z* 481.6 $[M + Na]^+$, and its molecular formula was established as $C_{30}H_{50}O_3$ from the $[M]^+$ peak at *m/z* 458.3747 (calcd 458.3760) in the HREIMS, indicating six degrees of unsaturation. The ^{1}H NMR spectrum (Table 2) exhibited a pair of proton resonances at δ_H 0.51 (d, J = 4.5 Hz) and 0.71 (d, J = 4.5 Hz) characteristic for a cyclopropane methylene, six tertiary methyls at δ_H 0.89, 0.96, 0.98, 1.04, 1.63, and 1.70, and one secondary methyl at δ_H 0.90 (3H, d, J = 6.5 Hz). Additionally, resonances for one olefinic proton at δ_H 5.11 (br t, J = 7.1 Hz) and three oxymethine protons at δ_H 3.52 (br s), 3.54 (br s), and 3.85 (br s) were observed. The ^{13}C NMR spectrum (Table 1) displayed signals for 30 carbons, including one methylene carbon at δ_C 29.1 ascribable to a cyclopropane ring, three oxymethine carbons at δ_C 67.9, 77.9, and 81.9, and two olefinic carbons at δ_C 125.2 and 130.9. The above ^{1}H and ^{13}C NMR data analysis suggested that **1** was a cycloartane-type triterpenoid bearing a double bond and three hydroxy groups.[36,37] The ^{1}H−^{1}H COSY, HMQC, and HMBC experiments (Figure 1) revealed that **1** shared the same planar structure with cycloartan-24-ene-1α,2α,3*β*-triol

* Corresponding author. Tel: 86-531-88382012. Fax: 86-531-88382019. E-mail: louhongxiang@sdu.edu.cn.

[†] Department of Natural Products Chemistry, School of Pharmaceutical Sciences.

[‡] Department of Biochemistry and Molecular Biology, School of Medicine.

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Table 1. ^{13}C NMR Spectroscopic Data of Compounds **1**–**9** (150 MHz in $CDCl_3$)

no.	**1**	**2**	**3**[a]	**4**	**5**	**6**[b]	**7** and **8**	**9**
1	77.9	75.8	77.8	75.8	73.7	76.0	75.3	75.3
2	67.9	71.6	71.9	71.6	36.6	73.1	72.5	72.5
3	81.9	80.5	77.8	80.2	73.8	77.7	77.2	78.1
4	39.7	40.0	40.8	40.0	40.5	40.8	40.1	40.1
5	33.4	38.9	41.5	38.9	39.5	39.8	39.0	39.3
6	20.5	20.6	21.4	20.6	20.8	21.1	20.6	20.6
7	25.4	25.5	25.9	25.4	25.8	25.9	25.5	25.6
8	47.6	47.9	47.5	47.9	48.0	48.0	47.9	47.9
9	20.4	20.4	21.2	20.3	20.8	20.0	19.9	20.3
10	29.6	29.4	30.3	28.7	30.3	30.0	28.7	29.0
11	26.0	26.1	27.3	26.0	26.1	26.1	25.7	26.1
12	32.7	32.7	33.7	32.6	32.8	32.9	32.6	32.7
13	45.1	45.2	45.9	45.1	45.1	45.4	45.0	45.1
14	48.8	48.8	49.8	48.7	48.7	49.1	48.6	48.8
15	35.6	35.7	36.2	35.7	35.7	35.8	35.5	35.7
16	28.1	28.1	28.7	28.1	28.1	28.2	27.9	28.1
17	52.2	52.3	53.0	52.2	52.3	52.3	52.1/52.0	52.2
18	18.0	18.1	18.3	18.1	18.2	18.3	18.0	18.1
19	29.1	29.7	28.5	29.4	30.0	29.3	29.3	29.4
20	35.9	35.9	36.7	35.8	35.9	36.7	35.8/35.6	35.9
21	18.2	18.2	18.6	18.2	18.2	18.5	18.0/18.1	18.2
22	36.3	36.3	37.1	36.3	36.3	39.7	32.6/32.4	36.3
23	24.9	24.9	25.5	24.9	24.9	128.5	25.5/25.4	24.9
24	125.2	125.2	126.0	125.2	125.2	137.1	64.8/68.6	125.2
25	130.9	130.9	131.3	130.9	131.0	81.1	58.0/58.3	131.0
26	17.6	17.7	17.7	17.6	17.6	25.1	18.4/18.5	17.7
27	25.7	25.7	25.9	25.7	25.7	25.3	24.7	25.7
28	19.3	19.4	19.4	19.4	19.4	19.4	19.2	19.4
29	26.0	25.6	26.3	25.5	25.1	26.5	25.5	25.6
30	20.3	15.3	14.9	15.4	13.0	15.1	14.2	14.2
AcO		172.8	170.2					
		21.2	21.3					
1′				175.0				
2′				43.8				
3′				29.7				
4′				22.4				
5′				22.5				

[a] Recorded in acetone-d_6. [b] Recorded in pyridine-d_5. Assignments were based on $^1H-^1H$ COSY, HMQC, and HMBC experiments and comparison with literature data.

(**9**).[27,38] The relative configuration of **1** was determined by analysis of its NOESY correlations (Figure 2a). Specifically, correlations of H-19a to H-1 and H_3-30; H-2 to H-3 and H_3-30; and H_3-29 to H-3 and H-5 unambiguously indicated that the hydroxy groups at C-1, C-2, and C-3 were all α-oriented. Therefore, the structure of compound **1** was assigned as cycloartan-24-ene-1α,2α,3α-triol, a C-3 epimer of compound **9**.

Compound **2** was isolated as a white, amorphous powder. Its molecular formula was determined to be $C_{32}H_{52}O_4$ based on the $[M]^+$ peak at *m*/*z* 500.3889 (calcd 500.3866) in the HREIMS. The IR spectrum of **2** showed an OH stretch at 3435 cm^{-1} and C═O absorption at 1720 cm^{-1}. The ^1H and ^{13}C NMR data of compound **2** were closely related to those of **9**,[27] except for additional signals arising from an acetoxy group, suggesting **2** to be a monoacetate of **9** (Tables 1 and 2). The proton signal at δ_H 2.14 (3H, s) and the carbon signals at δ_C 21.2 and 172.8 were assignable to the *O*-acetyl group. The H-3 resonance (δ_H 4.92) in compound **2** was downfield shifted ($\Delta\delta$ ca. 1.4) compared to that in **9**, indicating that the acetoxy group was at C-3 in **2**.[39] The location of the acetoxy group at C-3 was also verified by the HMBC correlations of H-3 to the acetyl carbonyl (δ_C 172.8), C-2, C-4, C-29, and C-30. Detailed assignments of the ^1H and ^{13}C NMR data were done on the basis of $^1H-^1H$ COSY, HMQC, and HMBC experiments and by comparison with those of **9**. The relative configuration of **2** was determined to be the same as that of **9** by the NOESY spectrum (Figure 2b). Thus, compound **2** was determined as 3β-acetoxycycloartan-24-ene-1α,2α-diol.

Compound **3** was isolated as colorless plates. The positive ESIMS exhibited a pseudomolecular ion at *m*/*z* 523.7 $[M + Na]^+$. Its molecular formula $C_{32}H_{52}O_4$ was deduced from the $[M]^+$ peak at *m*/*z* 500.3885 in the HREIMS (calcd 500.3866), which was identical to that of **2**. The similarity of the ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) of these two compounds suggested that **3** was an isomer of **2**. The only difference of these two compounds was that the acetoxy group in **3** was positioned at C-1 instead of C-3 as in **2**, which was confirmed by the long-range correlations of H-1 (δ_H 4.90) to the acetyl carbonyl (δ_C 170.2), C-2, C-3, C-5, and C-10 in the HMBC spectrum. On the basis of its 2D NMR data and comparison with that of **2**, compound **3** was identified to be 1α-acetoxycycloartan-24-ene-2α,3β-diol.

Compound **4** was obtained as white needles. Its molecular formula was established as $C_{35}H_{58}O_4$ by the HREIMS ($[M]^+$ at *m*/*z* 542.4341, calcd 542.4335). The ^1H and ^{13}C NMR data of **4** were similar to those of compound **2**, except for the absence of the C-3 acetoxy group, which was replaced by an isovaleroyloxy moiety as judged by the carbon signals at δ_C 175.0 (C-1′), 43.8 (C-2′), 29.7 (C-3′), 22.4 (C-4′), and 22.5 (C-5′), as well as the proton signals at δ_H 2.30 (2H, br d, J = 7.1 Hz, H_2-2′), 2.17 (m, H-3′), 1.03 (3H, d, J = 6.6 Hz, H_3-4′), and 1.03 (3H, d, J = 6.6 Hz, H_3-5′).[40] The HMBC correlations of H_2-2′/C-1′, C-3′, C-4′, and C-5′; H-3′/C-1′ C-2′, and C-4′; and H_3-4′/C-2′ and C-3′ confirmed the presence of the isovaleroyloxy group. In addition, the key HMBC correlation between H-3 (δ_H 4.94, d, J = 10.1 Hz) and C-1′ indicated that the isovaleroyloxy was attached to C-3. The relative configuration of **4** was determined to be the same as that of **2**. Accordingly, compound **4** was characterized as 3β-isovaleroyloxycycloartan-24-ene-1α,2α-diol.

Compound **5** was isolated as white plates, which showed a molecular ion peak at *m*/*z* 442.8 in the positive ESIMS. The HREIMS afforded a molecular ion peak at *m*/*z* 442.3830 corre-

Table 2. ^{1}H NMR Spectroscopic Data of Compounds **1–8** (600 MHz in $CDCl_3$)

no.	**1**	**2**	**3**[a]	**4**	**5**	**6**[b]	**7** and **8**	**9**
1	3.54 br s	3.53 br s	4.90 d (3.4)	3.54 br s	3.60 t (3.0)	3.91 d (2.7)	3.47 br s	3.56 br s
2	3.85 br s	3.73 d (10.1)	3.72 m	3.75 d (9.9)	1.76 m	4.14 dd (2.8,9.8)	3.59 br d (10.2)	3.65 br d (7.9)
					1.92 dd (3.2, 4.5)			
3	3.52 br s	4.92 d (10.1)	3.20 dd (3.7, 10.0)	4.94 d (10.1)	3.76 dd (4.5, 12.0)	4.11 d (9.8)	3.44 d (9.9)	3.50 d (9.8)
5	2.11 dd (4.4, 12.6)	2.04 dd (4.4, 12.2)	1.93 m	2.05 dd (4.0, 12.4)	1.90 m	2.40 dd (4.5, 12.6)	1.89 dd (4.6, 12.5)	1.94 dd (4.3, 12.8)
6α	0.86 m	0.87 m	0.92 m	0.84 m	0.84 m	0.88 m	0.83 m	0.85 m 0.85 m
6*β*	1.63 m	1.64 m	1.64 m	1.67 m	1.65 m	1.65 m	1.62 m	1.65 m
7α	1.18 m	1.17 m	1.18 m	1.18 m	1.12 m	1.33 m	1.11 m	1.16 m
7*β*	1.39 m	1.35 m	1.38 m	1.32 m	1.37 m	1.33 m	1.30 m	1.36 m
8	1.57 dd (4.9, 12.3)	1.53 dd (3.9, 12.1)	1.62 m	1.52 dd (3.8, 12.4)	1.50 dd (4.5, 12.5)	1.53 m	1.47 dd (4.2, 12.8)	1.53 dd (4.5, 10.2)
11α	2.27 m	2.29 m	1.91 m	2.30 m	2.18 m	2.67 m	2.30 m	2.29 m
11*β*	1.29 m	1.28 m	1.30 m	1.32 m	1.33 m	1.36 m	1.15 m	1.27 m
12	1.67 m	1.64 m	1.62 m	1.67 m	1.65 m	1.65 m	1.62 m	1.68 m
15	1.29 m	1.35 m	1.30 m	1.32 m	1.33 m	1.33 m	1.30 m	1.32 m
16α	1.92 m	1.90 m	1.91 m	1.90 m	1.90 m	1.92 m	1.89 m	1.91 m
16*β*	1.29 m	1.35 m	1.30 m	1.32 m	1.33 m	1.33 m	1.30 m	1.32 m
17	1.63 m	1.64 m	1.62 m	1.61 m	1.62 m	1.60 m	1.62 m	1.60 m
18	0.96 s	0.95 s	0.95 s	0.93 s	0.94 s	1.00 s	0.94 s	0.97 s
19a	0.51 d (4.5)	0.52 d (4.2)	0.60 d (4.7)	0.53 d (3.8)	0.48 d (4.6)	0.47 d (3.9)	0.45 d (3.9)	0.51 d (4.3)
19b	0.71 d (4.5)	0.72 d (4.2)	0.83 d (4.7)	0.74 d (3.9)	0.72 d (4.6)	0.70 d (3.9)	0.66 d (3.9)	0.73 d (4.3)
20	1.39 m	1.35 m	1.30 m	1.32 m	1.40 m	1.49 m	1.40 m	1.32 m
21	0.90 d (6.5)	0.89 br s	0.92 d (6.5)	0.89 br s	0.89 d (5.0)	0.88 d (6.4)	0.87 d (6.5)	0.90 d (6.4)
22	1.06 m	1.05 m	1.08 m	1.06 m	1.05 m	1.80 m	1.03 m	1.05 m
	1.43 m	1.42 m	1.45 m	1.44 m	1.43 m	2.27 br d (9.1)	1.62 m	1.42 m
23	1.89 m	1.90 m	1.91 m	1.90 m	1.90 m	5.84 m	1.40 m	1.88 m
	2.05 m	2.04 m	2.05 m	2.05 m	2.06 m		1.62 m	2.06 m
24	5.11 br t (7.1)	5.10 t (6.2)	5.12 t (7.2)	5.12 t (6.0)	5.11 br t (7.1)	5.97 d (15.9)	2.68 t (5.5)	5.11 t (7.0)
26	1.63 s	1.61 s	1.60 s	1.62 s	1.62 s	1.57 s	1.25 s	1.62 s
27	1.70 s	1.67 s	1.66 s	1.70 s	1.69 s	1.58 s	1.29 s	1.69 s
28	0.98 s	0.96 s	0.98 s	0.93 s	0.97 s	1.01 s	0.93 s	0.97 s
29	1.04 s	0.89 s	1.02 s	0.89 s	1.01 s	1.27 s	0.96 s	1.01 s
30	0.89 s	0.87 s	0.85 s	0.89 s	0.80 s	1.17 s	0.77 s	0.83 s
AcO		2.14 s	2.00 s					
2′				2.30 br d (7.1)				
3′				2.17 m				
4′				1.03 d (6.6)				
5′				1.03 d (6.6)				

[a] Recorded in acetone-d_6. [b] Recorded in pyridine-d_5. Chemical shifts are given in ppm. Figures in parentheses are coupling constants (J) in Hz. Assignments were based on ^{1}H−^{1}H COSY, HMQC, and HMBC experiments and comparison with literature data.

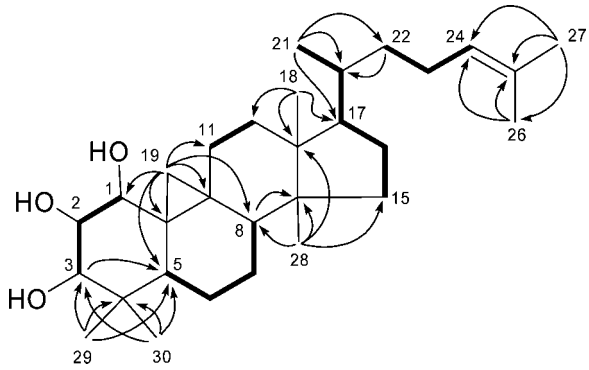

Figure 1. Key ^{1}H−^{1}H COSY (−) and HMBC (H→C) correlations of compound **1**.

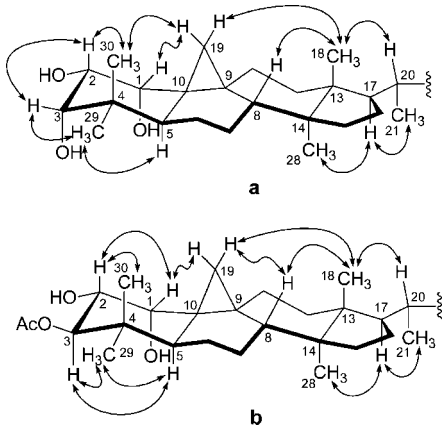

Figure 2. Key NOESY (↔) correlations of compounds **1** (a) and **2** (b).

sponding to a molecular formula of $C_{30}H_{50}O_2$ (calcd 442.3811), which was an oxygen atom less than that of **1** and **9**. The ^{1}H NMR spectrum of **5** was similar to that of **9**, except for the presence of a pair of methylene protons at δ_H 1.76 (m) and 1.92 (dd, J = 3.2, 4.5 Hz) instead of the oxymethine proton at δ_H 3.65 (br d, J = 7.9 Hz) in **9**,[27] suggesting that **5** possessed a cycloartane skeleton with two hydroxy groups. The positions of the two hydroxy groups at C-1 and C-3 were confirmed by ^{1}H−^{1}H COSY correlations of the protons at δ_H 1.76 (H-2a) to 3.60 (H-1) and 3.76 (H-3) and HMBC correlations from the proton at δ_H 3.76 (H-3) to the carbons at δ_C 36.6 (C-2), 40.5 (C-4), 25.1 (C-29), and 13.0 (C-30). The relative configuration of **5** was established by the NOESY experiment. On the basis of the comparison of its NMR spectroscopic data with those of **1** and 1α-acetoxy-9,19-cyclolanost-24-en-3β-ol,[4] the structure of compound **5** was deduced as cycloartan-24-ene-1α,3β-diol.

Compound **6** was obtained as a white, amorphous powder. The positive ESIMS exhibited a pseudomolecular ion at *m/z* 497.8 [M + Na]$^+$, consistent with the molecular formula $C_{30}H_{50}O_4$ as deduced by the HREIMS ([M − H_2O]$^+$ at *m/z* 456.3590; calcd for $C_{30}H_{48}O_3$, 456.3603). The ^{13}C NMR data (Table 1) of **6** resembled those of **9** except for the changes at δ_C 39.7 (C-22), 128.5 (C-23), 137.1 (C-24), 81.1 (C-25), and 25.1 (C-26) due to the replacement of $\Delta^{24,25}$ with $\Delta^{23,24}$ olefinic system and the presence of a hydroxy group at C-25. Correspondingly, two olefinic proton signals at δ_H 5.84 (m, H-23) and 5.97 (d, J = 15.9 Hz, H-24) were observed in the ^{1}H NMR spectrum (Table 2). The ^{1}H−^{1}H COSY coupling between proton signals at δ_H 1.49 (H-20) and 0.88 (H-21); 1.80 (H-22) and 5.84 (H-23); and 5.84 (H-23) and 5.97 (H-24), together with the HMBC correlations from proton signals at δ_H 5.97 (H-24) to the carbon resonances at δ_C 128.5 (C-23), 81.1 (C-25), 25.1 (C-26), and 25.3 (C-27) and δ_H 0.88 (H-21) to δ_C 52.3 (C-17), 36.7 (C-20), and 39.7 (C-22) (Figure 3a), confirmed the positions of the hydroxy group at C-25 and olefinic bond at C-23. A large vicinal

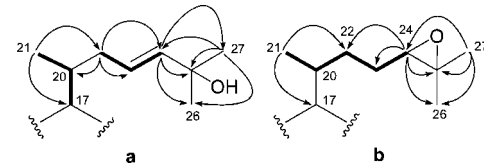

Figure 3. Key ^{1}H−^{1}H COSY (−) and HMBC (H→C) correlations for the side chain of compounds **6** (a) and **7** and **8** (b).

Table 3. Cytotoxicity of Compounds **1–9** against Human Prostate Cancer Cell Lines

	IC$_{50}$ values (μM)[a]	
compound	PC3	DU145
1	>50	>50
2	15.4 ± 3.53	20.7 ± 1.70
3	13.4 ± 0.78	>50
4	28.4 ± 2.40	>50
5	>50	>50
6	10.3 ± 0.33	34.7 ± 1.48
7 and **8**	13.8 ± 1.61	11.1 ± 1.06
9	10.1 ± 1.50	37.2 ± 2.50

[a] The results are means ± standard deviation of three independent replicates.

coupling constant value (J = 15.9 Hz) between H-23 and H-24 supported the *E*-type olefinic bond.[41–43] The relative configuration of **6** was established by the NOESY experiment, and the hydroxy groups at C-1, C-2, and C-3 were determined to be α-, α-, and β-oriented, respectively. Therefore, compound **6** was elucidated to be cycloartan-23*E*-ene-1α,2α,3β,25-tetrol.

Compounds **7** and **8** were obtained as an inseparable mixture of epimers in a ratio of 1:1. The molecular formula of **7** and **8** was determined to be $C_{30}H_{50}O_4$ by the HREIMS ([M]$^+$ at *m/z* 474.3724, calcd 474.3709), which was one oxygen atom more than **9**. The ^{13}C NMR spectrum showed a duplication of several signals that resonated at δ_C 64.8/64.6 (C-24), 58.3/58.0 (C-25), 52.1/52.0 (C-17), 35.8/35.6 (C-20), 32.6/32.4 (C-22), 25.5/25.4 (C-23), 18.5/18.4 (C-26), and 18.1/18.0 (C-21), suggesting the presence of two epimeric compounds. The proton signals in the ^{1}H NMR spectrum for the two epimers were similar to those of **9**.[27] The only difference was the observation of a triplet at δ_H 2.68 (t, J = 5.5 Hz) instead of the olefinic proton signal for H-24 in **9** at δ_H 5.11 (t, J = 7.0 Hz). Correspondingly, the C-24 and C-25 resonances were upfield shifted from δ_C 125.2 and 131.0 to δ_C 64.8/64.6 and 58.3/58.0, respectively. By comparison of the NMR data with those of **9** and 24, 25-epoxycycloartanols,[44] the planar structures of the epimers **7** and **8** were established as 24,25-epoxycycloartane-1,2,3-triol, which was verified by the ^{1}H−^{1}H COSY, HMQC, and HMBC spectra (Figure 3b). Similar to compounds **6** and **9**, the orientations of the hydroxy groups in the epimers were determined as 1α, 2α, and 3β, respectively. The signal duplications for C-17 and carbons of the side chains in the ^{13}C NMR spectrum of **7** and **8** were in agreement with differences of the ^{13}C NMR data reported for 24*R*,25- and 24*S*,25-epoxycycloartanols,[44] indicating that **7** and **8** were also C-24 epimers. Therefore, the structures of **7** and **8** were identified to be 24*R*,25-epoxycycloartane-1α,2α,3β-triol and 24*S*,25-epoxycycloartane-1α,2α,3β-triol, respectively.

The isolated compounds **1–9** were evaluated for their cytotoxicity against the PC3 and DU145 human prostate tumor cell lines using the MTT assay (Table 3). Compounds **2–4**, **6**, **9**, and the mixture of **7** and **8** exhibited moderate cytotoxic activity against PC3 cells with IC$_{50}$ values ranging from 10.1 to 28.4 μM, respectively. However, the test isolates were less active against DU145 cells, with IC$_{50}$ values ranging from 11.1 to 37.2 μM. Compounds **1** and **5** were inactive against both PC3 and DU145 cancer cell lines (IC$_{50}$ >50 μM).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. Melting points were determined on an X-6 melting-point apparatus (Beijing TECH Instrument Co. Ltd.) and were uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 600 spectrometer at 600 (^{1}H) and 150 (^{13}C) MHz, respectively. HREIMS and ESIMS spectra were measured on a Waters GCT system and an API 4000 mass spectrometer, respectively. Si gel (200–300 mesh) was used for column chromatography (CC). TLC was carried out with glass precoated Si gel GF_{254} plates (Qingdao Haiyang Chemical Co. Ltd.). Spots were detected using UV light or by spraying with 10% H_2SO_4−EtOH followed by heating.

Plant Material. The resin of *C. opobalsamum* was 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 ground into powder and extracted with petroleum ether (PE) in a Soxhlet apparatus for 36 h. The crude extract (290 g) was chromatographed over a Si gel column (10 cm i.d. × 45 cm) and eluted with a PE−EtOAc gradient (100% PE, 100:1, 100:5, 100:7, 100: 9,
100:17, 5:1, 10:3, 1:1, 100% EtOAc, each 30 L) as eluent to provide nine fractions (A−I). Fraction F (50 g) was subjected to a Si gel column (7 cm i.d. × 20 cm) and eluted with a PE−EtOAc gradient (97:3, 92: 8, 85:15, 7:3, 1:1, 1:100, each 12 L) to afford compound **9** (220 mg) and four subfractions (F1−F4). Compound **4** (9 mg) was obtained from fraction F1 (2.5 g) by a Si gel column (2.0 cm i.d. × 12 cm) eluted with PE−EtOAc−Et_2O (16:1:3) and further purified by preparative TLC with PE−$CHCl_3$−Et_2O (2:2:1). Fraction F2 (23 g) was subjected to a Si gel column (3.5 cm i.d. × 20 cm) eluted with a gradient of PE−Me_2CO to give **3** (80 mg) and another four subfractions (F21−F24). Compound **5** (64 mg) was isolated from fraction F22 (5.8 g) by a Si gel column (2.5 cm i.d. × 18 cm) eluted with PE−Me_2CO (88:12) and purified by recrystallization in PE−Me_2CO (1:1). Fraction F24 (4.3 g) was subjected to a Si gel column (2.0 cm i.d. × 25 cm) eluted with PE−Me_2CO (9:1) to afford compounds **1** (13 mg) and **2**. Compound **2** (10 mg) was further purified by a Si gel column (1.5 cm i.d. × 12 cm) eluted with PE−$CHCl_3$−CH_3OH (30:70:0.5) and preparative TLC with PE−Et_2O−Me_2CO (10:10:1). A mixture of **7** and **8** (9 mg) was obtained from fraction F3 (3.7 g) by a Si gel column (2.0 cm i.d. × 18 cm) and eluted with PE−Me_2CO (87:13) and then purified by preparative TLC with PE−EtOAc−Me_2CO (3:1:1) and a Si gel column (1.0 cm i.d. × 12 cm) eluted with $CHCl_3$−CH_3OH (100:2). Fraction F4 (8.9 g) was chromatographed over a Si gel column (3.5 cm i.d. × 20 cm) and eluted with PE−Me_2CO (8:2) to give **6** (45 mg), which was purified by preparative TLC with PE−Me_2CO (3:2).

Cycloartan-24-ene-1α,2α,3α-triol (1): white needles (PE−Me_2CO); mp 161–163 °C; $[\alpha]^{23}_D$ +62.0 (*c* 0.120, $CHCl_3$); IR (KBr) ν_{max} 3405, 2941, 2872, 1448, 1380, 1067 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HREIMS *m/z* 458.3747 $[M]^+$ (calcd for $C_{30}H_{50}O_3$, 458.3760); positive ESIMS *m/z* 481.6 $[M + Na]^+$ (100), 441.7 $[M + H - H_2O]^+$ (30), 423.7 $[M + H - 2H_2O]^+$ (30), 405.8 $[M + H - 3H_2O]^+$ (10).

3β-Acetoxycycloartan-24-ene-1α,2α-diol (2): white, amorphous powder; $[\alpha]^{23}_D$ +30.0 (*c* 0.115, $CHCl_3$); IR (KBr) ν_{max} 3435, 2940, 2872, 1720, 1467, 1373, 1261, 1084, 1043, 803 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HREIMS *m/z* 500.3889 $[M]^+$ (calcd for $C_{32}H_{52}O_4$, 500.3866); positive ESIMS *m/z* 523.7 $[M + Na]^+$ (100), 518.7 $[M + H_2O]^+$ (50), 483.7 $[M + H - H_2O]^+$ (10), 465.6 $[M + H - 2H_2O]^+$ (25), 315.2 (25), 239.4 (50).

1α-Acetoxycycloartan-24-ene-2α,3β-diol (3): colorless plates (acetone); mp 97–99 °C; $[\alpha]^{23}_D$ +35.0 (*c* 0.115, $CHCl_3$); IR (KBr) ν_{max} 3417, 2943, 2871, 1726, 1374, 1246, 1069 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HREIMS *m/z* 500.3885 $[M]^+$ (calcd for $C_{32}H_{52}O_4$, 500.3866); positive ESIMS *m/z* 523.7 $[M + Na]^+$ (20), 518.7 $[M + H_2O]^+$ (96), 458.7 (5), 441.7 (100), 423.6 (95), 405.8 (50).

3β-Isovaleroyloxycycloartan-24-ene-1α,2α-diol (4): white needles (PE-Me_2CO); mp 68–70 °C; $[\alpha]^{23}_D$ +33.0 (*c* 0.120, $CHCl_3$); IR (KBr) ν_{max} 3443, 2959, 2871, 1716, 1468, 1370, 1295, 1260, 1193, 1099 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HREIMS *m/z* 542.4341 $[M]^+$ (calcd for $C_{35}H_{58}O_4$, 542.4335); positive ESIMS *m/z* 565.8 $[M + Na]^+$ (100), 525.7 $[M + H - H_2O]^+$ (30), 507.6 $[M + H - 2H_2O]^+$ (65).

Cycloartan-24-ene-1α,3β-diol (5): white plates (PE−Me_2CO); mp 140–141 °C; $[\alpha]^{23}_D$ +58.0 (*c* 0.145, $CHCl_3$); IR (KBr) ν_{max} 3446, 2947, 2869, 1449, 1375, 1044 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HREIMS *m/z* 442.3830 $[M]^+$ (calcd for $C_{30}H_{50}O_2$, 442.3811); positive ESIMS *m/z* 442.8 $[M]^+$ (12), 425.7 $[M + H - H_2O]^+$ (100), 407.8 $[M + H - 2H_2O]^+$ (7).

Cycloartan-23*E*-ene-1α,2α,3β,25-tetrol (6): white, amorphous powder; $[\alpha]^{23}_D$ +48.0 (*c* 0.110, CH_3OH); IR (KBr) ν_{max} 3396, 2933, 2869, 1457, 1375, 1074 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HREIMS *m/z* 456.3590 $[M - H_2O]^+$ (calcd for $C_{30}H_{48}O_3$, 456.3603); positive ESIMS *m/z* 497.8 $[M + Na]^+$ (25), 492.7 $[M + H_2O]^+$ (100), 475.6 $[M + H]^+$ (30), 457.8 $[M + H - H_2O]^+$ (50), 439.8 $[M + H - 2H_2O]^+$ (90), 421.7 $[M + H - 3H_2O]$ (31).

Epimeric mixture of 24,25-epoxycycloartane-1α,2α,3β-triol (7 and 8): white, amorphous powder; $[\alpha]^{23}_D$ +49.0 (*c* 0.140, $CHCl_3$); IR (KBr) ν_{max} 3431, 2943, 2871, 1467, 1377, 1099, 1076 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2; HREIMS *m/z* 474.3724 $[M]^+$ (calcd for $C_{30}H_{50}O_4$, 474.3709); positive ESIMS *m/z* 497.4 $[M + Na]^+$ (76), 457.7 $[M + H - H_2O]^+$ (75), 439.7 $[M + H - 2H_2O]^+$ (100), 421.6 $[M + H - 3H_2O]^+$ (65).

Cytotoxicity Assay. The MTT assay was used to measure cell inhibition of tested compounds in 96-well plates.[45] The cells were treated with the test compounds for 48 h. After addition of MTT (10 μL/well, 5 mg/mL in phosphated-buffered saline), the plates were incubated for 2 h under 5% CO_2 at 37 °C. Then, the absorbance was determined at 570 nm.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **1**–**8**; 2D NMR spectra of compounds **1**–**8**; IR and MS spectra of compounds **1**–**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Vollesen, K. In *Flora of Ethiopia*; Hedberg, I., Edwards, S., Eds.; Addis Ababa University Press: Addis Ababa, 1989; Vol. 3, pp 442–478.
(2) Zhu, N.; Kikuzaki, H.; Sheng, S.; Sang, S.; Rafi, M. M.; Wang, M.; Nakatani, N.; DiPaola, R. S.; Rosen, R. T.; Ho, C. T. *J. Nat. Prod.* **2001**, *64*, 1460–1462.
(3) Zhu, N.; Sheng, S.; Sang, S.; Rosen, R. T.; Ho, C. T. *Flavour Fragr. J.* **2003**, *18*, 282–285.
(4) Provan, G. J.; Waterman, P. G. *Phytochemistry* **1988**, *27*, 3841–3843.
(5) Dekebo, A.; Dagne, E.; Hansen, L. K.; Gautun, O. R.; Arne, J.; Aasen, A. J. *Phytochemistry* **2002**, *59*, 399–403.
(6) Patil, V. D.; Nayak, U. R.; Sukh, D. *Tetrahedron* **1972**, *28*, 2341–2352.
(7) Patil, V. D.; Nayak, U. R.; Sukh, D. *Tetrahedron* **1973**, *29*, 341–348.
(8) Patil, V. D.; Nayak, U. R.; Sukh, D. *Tetrahedron* **1973**, *29*, 1595–1598.
(9) Fourie, T. G.; Snyckers, F. O. *J. Nat. Prod.* **1989**, *52*, 1129–1131.
(10) Fatope, M. O.; Al-Burtomani, S. K. S.; Ochei, J. O.; Abdulnour, A. O.; Al-Kindy, S. M. Z.; Takeda, Y. *Phytochemistry* **2003**, *62*, 1251–1255.
(11) Zhu, N.; Rafi, M. M.; Dipaola, R. S.; Xin, J.; Chin, C. K.; Badmaev, V.; Ghai, G.; Rosen, R. T.; Ho, C. T. *Phytochemistry* **2001**, *56*, 723–727.
(12) Brieskorn, C. H.; Noble, P. *Tetrahedron Lett.* **1980**, *21*, 1511–1514.
(13) Brieskorn, C. H.; Noble, P. *Phytochemistry* **1983**, *22*, 187–189.
(14) Brieskorn, C. H.; Noble, P. *Phytochemistry* **1983**, *22*, 1207–1211.
(15) Thomas, A. F. *Tetrahedron* **1961**, *16*, 212–216.
(16) Thomas, A. F.; Heusler, K.; Müller, J. M. *Tetrahedron* **1961**, *16*, 264–270.
(17) Thomas, A. F.; Willhalm, B. *Tetrahedron Lett.* **1964**, *5*, 3177–3183.
(18) Dekebo, A.; Lang, M.; Polborn, K.; Dagne, E.; Steglich, W. *J. Nat. Prod.* **2002**, *65*, 1252–1257.
(19) Waterman, P. G.; Ampofo, S. *Phytochemistry* **1985**, *24*, 2925–2928.
(20) Manguro, L. O.; Ugi, I.; Lemmen, P. *Chem. Pharm. Bull* **2003**, *51*, 483–486.
(21) Dekebo, A.; Dagne, E.; Hansen, L. K.; Gautun, O. R.; Aasen, A. J. *Tetrahedron Lett.* **2000**, *41*, 9875–9878.
(22) Dekebo, A.; Dagne, E.; Sterner, O. *Fitoterapia* **2002**, *73*, 48–55.

(23) Ashry, E. S. H. E.; Rashed, N.; Salama, O. M.; Saleh, A. *Pharmazie* **2003**, *58*, 163–168.
(24) Hanuš, L. O.; Řezanka, T.; Dembitsky, V. M.; Moussaieff, A. *Biomed. Papers* **2005**, *149*, 3–28.
(25) Habtemariam, S. *Toxicon* **2003**, *41*, 723–727.
(26) Shoemaker, M.; Hamilton, B.; Dairkee, S. H.; Cohen, I.; Campbell, M. J. *Phytother. Res.* **2005**, *19*, 649–651.
(27) Shen, T.; Wan, W.; Yuan, H.; Kong, F.; Guo, H.; Fan, P.; Lou, H. *Phytochemistry* **2007**, *68*, 1331–1337.
(28) Dolara, P.; Luceri, C.; Ghelardini, C.; Monserrat, C.; Aioli, S.; Luceri, F.; Lodovici, M.; Menichetti, S.; Romanelli, M. N. *Nature* **1996**, *4*, 29.
(29) Meselhy, M. R. *Phytochemistry* **2003**, *62*, 213–218.
(30) Francis, J. A.; Raja, S. N.; Nair, M. G. *Chem. Biodiversity* **2004**, *1*, 1842–1853.
(31) Matsuda, H.; Morikawa, T.; Ando, S.; Oominami, H.; Murakami, T.; Kimura, I.; Yoshikawa, M. *Bioorg. Med. Chem.* **2004**, *12*, 3037–3046.
(32) Saeed, M. A.; Sabir, A. W. *Fitoterapia* **2004**, *75*, 204–208.
(33) Dolara, P.; Corte, B.; Ghelardini, C.; Pugliese, A. M.; Cerbai, E.; Menichetti, S.; Nostro, A. L. *Planta Med.* **2000**, *66*, 356–358.
(34) Meeker, H. G.; Linke, H. A. B. *Compend. Contin. Educ. Dent.* **1988**, *9*, 32–41.
(35) Yuan, H.; Pan, Y.; Young, C. Y. F. *Cancer Lett.* **2004**, *213*, 155–163.
(36) Cantillo-Ciau, Z.; Brito-Loeza, W.; Quijano, L. *J. Nat. Prod.* **2001**, *64*, 953–955.
(37) Hossain, C. F.; Jacob, M. R.; Clark, A. M.; Walker, L. A.; Nagle, D. G. *J. Nat. Prod.* **2003**, *66*, 398–400.
(38) Nes, W. D.; Koike, K.; Jia, Z.; Sakamoto, Y.; Satou, T.; Nikaido, T.; Griffin, J. F. *J. Am. Chem. Soc.* **1998**, *120*, 5970–5980.
(39) Wang, X.-N.; Fan, C.-Q.; Yue, J.-M. *Steroids* **2006**, *71*, 720–724.
(40) Ribeiro, A.; Pilo-Veloso, D.; Howarth, O.; Zani, C. L. *Org. Lett.* **1999**, *1*, 1897–1900.
(41) Takahashi, S.; Satoh, H.; Hongo, Y.; Koshino, H. *J. Org. Chem.* **2007**, *72*, 4578–4581.
(42) Weber, S.; Puripattanavong, J.; Brecht, V.; Frahm, A. W. *J. Nat. Prod.* **2000**, *63*, 636–642.
(43) Luo, H.-F.; Li, Q.; Yu, S.; Badger, T. M.; Fang, N. *J. Nat. Prod.* **2005**, *68*, 94–97.
(44) Greca, M. D.; Fiorentino, A.; Monaco, P.; Previter, L. *Phytochemistry* **1994**, *35*, 1017–1022.
(45) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.

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