Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2021, **19**(9): 686-692
doi: 10.1016/S1875-5364(21)60099-7
Chinese Journal of Natural Medicines

•Research article•

New tirucallane-type triterpenoids from the resin of *Boswellia carterii* and their NO inhibitory activities

LIU Fang-Shen[1, 2Δ], ZHANG Ting-Ting[1, 2Δ], XU Jun[1, 2], JING Qin-Xue[1, 2], GONG Chi[1, 2], DONG Bang-Jian[3], LI Da-Hong[1, 2], LIU Xiao-Qiu[2], LI Zhan-Lin[1, 2], YUAN Zhong[2*], HUA Hui-Ming[1, 2*]

[1] *Key Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, China;*
[2] *School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China;*
[3] *State Key Laboratory of Medicinal Chemical Biology, College of Pharmacy, and Tianjin Key Laboratory of Molecular Drug Research, Nankai University, Tianjin 300071, China*

Available online 20 Sep., 2021

[ABSTRACT] Six new tirucallane-type triterpenoids (**1**−**6**), along with ten known triterpenoids, were isolated from methylene chloride extract of the resin of *Boswellia carterii* Birdw. By the application of the comprehensive spectroscopic data, the structures of the compounds were clarified. The experimental electronic circular dichroism spectra were compared with those calculated, which allowed to assign the absolute configurations. Compounds **5** and **6** possessed a 2, 3-*seco* tirucallane-type triterpenoid skeleton, which were first reported. Their inhibitory activity against NO formation in LPS-activated BV-2 cells were evaluated. Compound **9** showed appreciable inhibitory effect, with an IC_{50} value of 7.58 ± 0.87 μmol·L^{-1}.

[KEY WORDS] *Boswellia carterii* Birdw.; *seco*-Tirucallane triterpenoids; NO inhibition
[CLC Number] R284, R965 **[Document code]** A **[Article ID]** 2095-6975(2021)09-0686-07

Introduction

The arbor of the *Boswellia* species (Burseraceae), derived from Somalia, India, Arabic Peninsula and Ethiopia, produce a kind of gum resin which is referred to frankincense or olibanum [1]. Olibanum was used to treat rheumatoid arthritis, osteoarthritis, dysmenorrhea, gout, ulcers, as well as swelling and pain caused by injuries in traditional Chinese and Ayurvedic medicine for a long time [2-4]. Previous researches showed that the extract and constituents of olibanum exhibited anti-inflammatory, cytotoxic, and antitumor activities [5-8]. Boswellic acids (the total triterpene acids of olibanum) exerted anti-inflammatory activity by inhibiting the activity of 5-lipoxygenase and reducing the synthesis of leukotriene [9]. Thus, a series tetracyclic (tirucallane-, and lanostane-type), pentacyclic (oleanane-, ursane-, and lupane-type) triterpenoids, as well as diterpenoids (cembrane-, prenylaromadendrane-, verticillane-, and prenylmaaliane-type) and monoterpenoids were isolated during phytochemical research on olibanum [10-18]. Among them, olibanumol A (monoterpene), olibanumol D (diterpene), and olibanumol E (triterpene), were reported to inhibit NO formation stimulated by LPS in mouse peritoneal macrophages, and thus exhibited potential anti-inflammatory activity [12-14]. In our continuous effort to search for natural anti-inflammatory agents [19-20], six new tirucallane-type triterpenoids (**1**−**6**) along with ten known compounds (**7**−**16**) were obtained (Fig. 1). Moreover, through 1D and 2D NMR spectroanalysis, their structures were fully determined. The electronic circular dichroism (ECD) method was used in structural research to define their absolute configurations. The inhibitory activities against NO formation in LPS-activated BV-2 cell were evaluated.

Results and Discussion

Compound **1** was obtained as colorless needles with spe-

[Received on] 26-Jun.-2021
[Research funding] This work was supported by the National Natural Science Foundation of China (No. 81973465), the National Science and Technology Major Project of the Ministry of Science and Technology of China (No. 2018ZX09735005) and the Key Laboratory Basic Research Projects of the Department of Education in Liaoning Province, China (No. LZ2014044).
[*] **Corresponding author]** E-mails: yuanzhong@syphu.edu.cn (YUAN Zhong); huimhua@163.com (HUA Hui-Ming)
[Δ] These authors contributed equally to this work.
These authors have no conflict of interest to declare.
Dedicated to the 90th Anniversary of the Founding of Shenyang Pharmaceutical University

cific optical rotation of $[\alpha]_D^{20}$ −31.2 (*c* 0.14, MeOH), and the molecular formula was determined to be $C_{30}H_{46}O_5$, based on HR-ESIMS which gave a negative-ion peak at *m/z* 485.3272. Interpretation of the information in its ^{1}H NMR (Table 1) suggested the signals were consistent with one olefinic proton (δ_H 5.96), two oxygenated methines (δ_H 5.09, 3.64), and seven methyls (δ_H 1.63, 1.58, 1.56, 1.47, 1.12, 1.07, and 0.97). According to its ^{13}C NMR (Table 2) and HSQC spectra, thirty carbon resonances were deduced, which were attributable to a trisubstituted double bond (δ_C 124.7 and 131.6), an *α*, *β*-unsaturated ketone (δ_C 199.9, 139.7 and 164.4), two oxygenated methines (δ_C 74.0 and 67.2), and one carboxylic group (δ_C 178.6). In addition to the above groups, other carbons were resolved as seven methyls, eight methylenes, three methines, and four quaternary carbons. The above data disclosed that compound **1** was a tirucallane-type triterpene [21]. Furthermore, two hydroxyls attached to C-3 and C-11 respectively were defined by the existence of correlations of H_3-28 (δ_H 1.12) with C-4, C-5, and C-29; H_3-29 (δ_H 0.97) with C-3, C-4, and C-5; H-3 (δ_H 3.64) with C-1, and C-5; and H-11 (δ_H 5.09) with C-8, C-9, and C-12. Moreover, the HMBC correlations from H_3-19 (δ_H 1.56) to C-1, C-5, C-9 and C-10; H_3-27 (δ_H 1.58, s) to C-25 and C-26; H_3-30 (δ_H 1.47) to C-8, C-13, C-14, and C-15; H-5 (δ_H 2.67) to C-4, C-6, C-7, C-9, C-10, and C-19 were observed, which confirmed the presence of the ketone at C-7, and the existence of two double bonds at C8−C9 and C24−C25, respectively. The planar structure of **1** was delineated as shown, on account of the above data. The levorotatory optical rotation was consistent with those of known tirucallanes [21]. The NOE correlations (Fig. S1) of H_3-19 with H_3-30 and H_3-29, H_3-18 with H-16*α*, as well as H-17 with H_3-30 assembled the tirucallane-type skeleton. The correlations of H-11/H_3-18 and H-3/H_3-29 demonstrated H-11 on the *α*-face and H-3 on the *β*-face. The

Table 1 ^{1}H NMR data for 1−6 (C_5D_5N, δ in ppm, *J* in Hz)

NO.	1 [a]	2 [a]	3 [a]	4 [b]	5 [a]	6 [a]
1	2.69, m 2.57, m	3.09, m 1.92, m	1.73, m 1.44, m	1.87, m 1.66, m	2.88, d (14.7) 2.84, d (14.7)	2.93, s
2	2.17, m 1.91, m	2.92, dt (14.8, 5.5) 2.43, m	2.50, m	2.34, m 2.29, m		
3	3.64, brs					
5	2.67, m	2.31, dd (14.0, 4.3)	1.67, dd (12.5, 1.7)	1.68, m	3.43, t (4.8)	3.44, t (4.9)
6	2.67, m	2.75, dd (18.4, 14.0) 2.56, dd (18.4, 4.3)	1.53, m 1.34, m	2.00, m 1.75, m	2.33, m 1.98, m	2.31, m 1.96, m
7			1.57, m 1.46, m	5.40, brs	2.33, m 2.00, m	2.18, m
11	5.09, t (8.2)	4.87, t (8.2)	1.89, m 1.25, m	5.16, brs	2.07, m 1.48, m	2.16, m 2.03, m
12	2.78, m 2.40, m	2.81, dd (12.7, 8.4) 2.40, m	1.93, m	2.44, brs 1.95, m	2.12, m 1.98, m	2.00, m 1.84, m
15	2.51, m 1.76, m	2.51, m 1.77, m	1.58, m 1.24, m	2.31, m 2.08, m	1.71, m 1.29, t (10.2)	1.71, m 1.31, t (9.9)
16	2.05, m 1.40, m	2.08, m 1.44, m	2.03, m 1.88, m	1.40, m 1.39, m	2.27, m 2.37, m	1.86, m 1.71, m
17	2.51, m	2.52, m	2.48, m	2.55, m	2.44, dd (9.1)	2.58, m
18	1.07, s	1.12, s	1.06, s	1.00, s	1.18, s	0.94, s
19	1.56, s	1.66, s	0.92, s	1.09, s	1.51, s	1.58, s
20	2.51, m	2.62, t (11.2)	2.68, td (10.8, 2.8)	2.67, td (11.1, 3.2)	2.64, td (11.1, 3.2)	2.86, m
22	2.32, m 2.21, m	2.36, m 2.27, m	2.48, m 2.03, m	2.48, m 2.03, m	2.17, m 2.06, m	2.31, m 1.86, m
23	1.90, m 1.74, m	1.92, m 1.75, m	2.32, m 1.89, m	2.32, m 1.31, m	1.93, m 1.77, m	5.13, m
24	5.28, t (6.9)	5.31, d (7.0)	3.83, d (8.9)	3.84, d (8.6)	5.32, t (7.0)	5.30, d (8.7)
26	1.63, s	1.65, s	1.50, s	1.51, s	1.66, s	1.68, s
27	1.58, s	1.60, s	1.46, s	1.45, s	1.61, s	1.67, s
28	1.12, s	1.11, s	1.15, s	1.10, s	1.49, s	1.51, s
29	0.97, s	1.08, s	1.04, s	1.04, s	1.46, s	1.49, s
30	1.47, s	1.43, s	0.94, s	0.96, s	1.01, s	1.01, s

a: ^{1}H NMR data were recorded at 600 MHz; b: ^{1}H NMR data were recorded at 400 MHz

Table 2 ^{13}C NMR data for **1**−**6** (C_5D_5N, 100 MHz, δ in ppm)

NO.	1	2	3	4	5	6
1	27.9	34.3	35.5	36.9	45.9	45.9
2	26.6	34.8	34.6	35.0	174.2	174.3
3	74.0	213.8	216.4	214.8	182.5	182.5
4	38.2	47.5	47.1	47.7	45.7	45.7
5	43.7	50.8	51.4	50.3	43.7	43.8
6	36.2	36.6	20.3	24.3	20.6	20.6
7	199.9	198.7	27.1	118.9	22.7	22.8
8	139.7	140.0	134.6	141.5	135.2	135.3
9	164.4	162.4	133.0	144.1	131.6	131.8
10	40.2	39.9	37.2	36.4	41.3	41.4
11	67.2	67.5	21.4	116.5	24.3	24.4
12	41.4	41.1	29.1	36.3	29.4	29.5
13	46.5	46.4	44.4	44.4	44.6	44.8
14	48.1	48.2	49.9	49.6	50.3	49.8
15	32.4	32.3	29.7	30.5	30.4	30.8
16	27.0	27.0	27.5	27.1	26.6	24.0
17	46.6	46.5	47.5	48.2	47.4	44.8
18	16.6	16.8	16.1	16.6	16.2	17.8
19	19.5	18.8	19.6	19.9	22.0	22.2
20	48.7	48.8	49.7	49.6	48.8	41.8
21	178.6 [a]	178.6 [a]	179.0	178.8 [a]	178.5	178.9
22	26.4	26.6	31.0	31.1	27.3	34.9
23	33.2	33.2	30.5	29.9	33.2	75.2
24	124.7	124.8	79.4	79.4	124.8	124.1
25	131.6	131.6	72.6	72.5	131.6	139.0
26	25.7	25.7	25.8	25.9	25.7	25.5
27	17.6	17.6	25.8	25.8	17.6	18.1
28	28.3	24.7	26.7	24.7	23.5	23.7
29	22.1	21.6	21.1	22.0	28.4	28.5
30	25.5	25.6	24.3	23.2	23.5	23.6

[a] The signal was observed in C_5D_5N + CF_3COOH

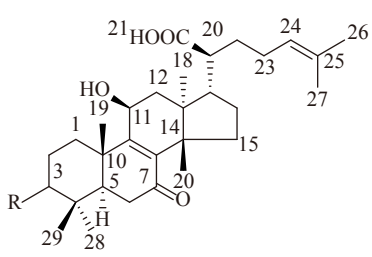
small coupling constants of H-3 and H_2-2 also proved the above deduction. To clarify the absolute configuration, we used ECD calculations which demonstrated that experimental ECD spectrum of **1** matched the calculated one of **1a** (Fig. S2), suggesting the absolute configuration (3*R*, 5*R*, 10*S*, 11*S*, 13*S*, 14*S*, 17*S*, 20*S*).

Compound **2**, colorless needles, had the molecular formula $C_{30}H_{44}O_5$, which was corresponded to the molecular ion peak in the HR-ESIMS at *m/z* 483.3102 $[M - H]^-$ (Calcd. 483.3116 for $C_{30}H_{43}O_5$). Due to the main difference between compounds **1** and **2** that a ketone signal (δ_C 213.8) existed in **2** and an oxygenated methine (δ_H 3.64, δ_C 74.0) in **1**, it was possible to indicate that compound **2** was a C-3 oxidized derivative of **1**. Further correlation between H-2 and the ketone carbon confirmed the carbonyl at C-3, which was also proved by the deshielded resonance of C-2 in **2** with respect to that in **1** (δ_C 34.8 in **2**; δ_C 26.6 in **1**). By examining the NOE spectrum, a conclusion was drawn that the relative configuration of **2** was identical to that of **1**. The absolute configuration of **2** was determined as (5*R*, 10*S*, 11*S*, 13*S*, 14*S*, 17*S*, 20*S*) by ECD calculations.

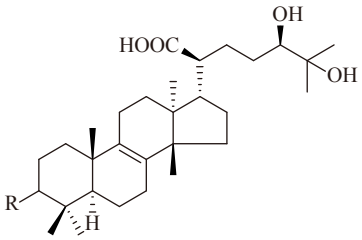
Compound **3**, white amorphous powder, had the molecular formula $C_{30}H_{48}O_5$, according to its HR-ESIMS spectrum at *m/z* 487.3406 $[M - H]^-$. ^{1}H NMR data (Table 1) revealed the signals were attributable to seven tertiary methyl groups and an oxygenated methine (δ_H 3.83). Moreover, ^{13}C NMR spectrum (Table 2) revealed that thirty carbons were attributable to a ketone carbonyl carbon (δ_C 216.4), one carboxylic group (δ_C 179.0), two olefinic carbons (δ_C 134.6, 133.0), and two oxygenated sp^3 carbons (δ_C 79.4, 72.6). Comparing its NMR data with 3*α*, 24*R*, 25-trihydroxytirucalla-8-en-21-oic acid (**7**) [21], it was indicated that **3** was a C-3 oxidized derivative of **7**. The ketone carbonyl unit was identified to exist at the position of C-3 by the existence of HMBC correlations from H_3-28 and H_3-29 to C-3, and from H-2 to C-1, C-3, and C-10. Furthermore, NOESY correlations of H_3-19/H_3-29, and H-17/H_3-30 with *β*-orientation, as well as H_3-28/H-5, H-15*α*/H_3-18, and H_3-18/H-20 assigned *α*-orientation, confirmed that the relative configuration of **3** was the same as the tirucallane skeleton. Finally, the absolute configuration of C-24 was established by the method of $Mo_2(OAc)_4$-induced ECD [22-24] for vicinal diols. The methyl ester (**3a**) of **3** exhibited the positive Cotton effect at 310 nm, which demonstrated a 24*R* absolute configuration for **3**. As a result of the above information, compound **3** was identified.

HR-ESIMS of **4** provided a molecular formula as $C_{30}H_{46}O_5$, based on the ion peak at *m/z* 485.3269 $[M - H]^-$. NMR signals (Table 1) displayed resonances for seven tertiary methyls (δ_H 1.51, 1.45, 1.10, 1.09, 1.04, 1.00, and 0.96), two olefinic protons (δ_H 5.40, 5.16), and an oxygenated methine (δ_H 3.84). The characteristic signals also disclosed a ketone carbonyl (δ_C 214.8), two conjugated double bonds (δ_C 144.1, 141.5, 118.9, 116.5), as well as two oxygenated carbons (δ_C 79.4 and 72.5). The NMR data showed similarity to **3**, except for possessing one more double bond and two olefinic protons than **3**. Furthermore, correlations of H_3-19 with C-9 and C-10, H-12*β* with C-9, C-11, C-14, and C-18, H_3-30 with C-8, C-13, and C-14, and of H-7 with C-9 in the HMBC, determined the conjugated double bond positions at C7−C8 and C9−C11. The aforementioned analysis defined the structure of **4** as 24*R*, 25-dihydoxy-3-oxo-tirucalla-7, 9(11)-dien-21-oic acid.

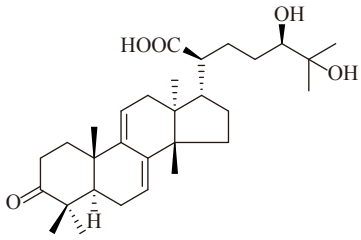
Compound **5** was defined to be $C_{30}H_{46}O_6$, based on its HR-ESIMS (*m/z* 525.3193 $[M + Na]^+$). Further detailed ^{1}H NMR spectrum of **5** confirmed the existence of seven methyl



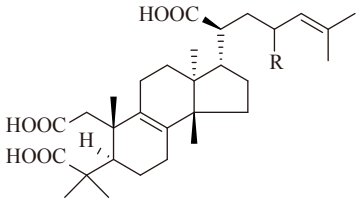
1 R = *α*-OH
2 R = ═O



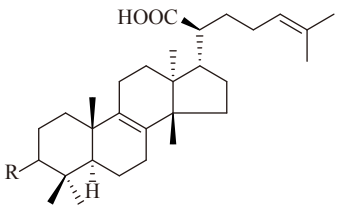
3 R = ═O
7 R = *α*-OH



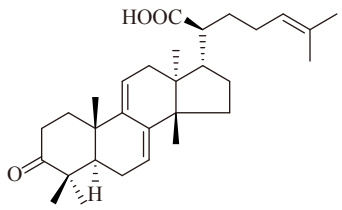
4



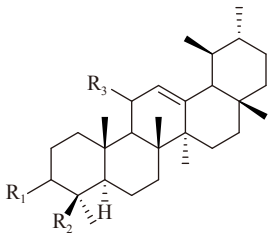
5 R = H
6 R = *β*-OH



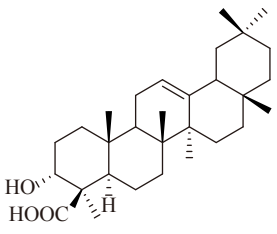
8 R = *α*-OH
9 R = ═O



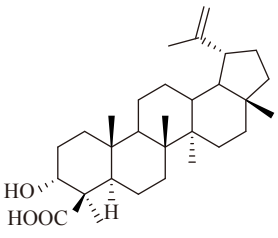
10



11 R_1 = *β*-OH, R_2 = CH_3, R_3 = H
12 R_1 = *α*-OH, R_2 = COOH, R_3 = H
13 R_1 = *α*-OAc, R_2 = COOH, R_3 = H
14 R_1 = *α*-OAc, R_2 = COOH, R_3 = ═O



15



16

Fig. 1 Structures of compounds 1−16

singlets (δ_H 1.66, 1.61, 1.51, 1.49, 1.46, 1.18 and 1.01), and an olefinic proton (δ_H 5.32). Likewise, thirty carbon signals, some of which were attributed to four olefinic carbons at δ_C 135.2, 131.6, 131.6, 124.8, and three carboxyls at δ_C 182.5, 178.5, 174.2, were determined by ^{13}C NMR. By contrasting the NMR data with those of **8** [25], it was inferred that compound **5** might be a 2,3-*seco* tirucallane triterpene. Moreover, the HMBC data of **5** determined two double bonds respectively at C8−C9 and C24−C25, as for the correlations from H_3-19 to C-5, C-9, and C-10, from H_3-30 to C-8, C-13, and C-14, and from H_3-26 and H_3-27 to C-24 and C-25. In combination with a carboxyl group located at C-21 which was ascertained by the correlations from H-20 to C-21, the above mentioned information established the structures of rings B-D and C-17 side chain moiety. Thus, those remaining signals of a methylene (δ_C 45.9), two carboxylic groups (δ_C 182.5, 174.2), a quaternary carbon (δ_C 45.7) as well as two methyls (δ_C 23.5, δ_C 28.4) were assigned to ring A of **5**. These data, along with the HR-ESIMS data, supported a *seco*-ring skeleton of **5**. The HMBC gave the correlations of H_2-1 with C-2, C-5, C-9, C-10, and C-19, H_3-28 with C-3, C-5 and C-29, H_3-29 with C-28, and of H-5 with C-3, C-4, C-28, and C-29 which supported **5** as a 2,3-*seco* derivative of a tirucallane-type triterpene with 2,3-dicarboxyl groups. Significant NOE correlations confirmed the relative configuration of **5** as shown in Fig. S3. The correlations of H-5/H_2-1, H_3-19/H_3-29, H_3-18/H-15*α*, H_3-30/H-15*β*, and H_3-30/H-17 indicated that H-17, CH_3-19, and CH_3-30 were *β*-oriented, and H-5, CH_3-18 and C-17 side chain were *α*-oriented. We compared the experimental ECD spectrum with calculated ones (Fig. S4) to clarify the absolute configuration of **5**. A conclusion was obtained that of **5** was defined as (5*R*, 10*S*, 13*S*, 14*S*, 17*S*, 20*S*).

Compound **6**, colorless needles, possessed the molecular formula $C_{30}H_{46}O_7$. The HR-ESIMS displayed a negative ion peak at *m/z* 499.3060 $[M - H_2O - H]^-$. Comparing its ^{13}C NMR of **6** with those of **5**, it was found that **6** possessed one hydroxyl group at C-23, supported by the correlations of H-24 with C-23, C-26, and C-27 and the deshielded signal of C-

22 in **6** (δ_C 34.9) with respect to that in **5** (δ_C 27.3). Its ^{1}H NMR data revealed seven methyl singlets at δ_H 1.68, 1.67, 1.58, 1.51, 1.49, 1.01 and 0.94, and an olefinic proton (δ_H 5.30). The absolute configuration of the skeleton of **6** was same to that of **5**, with respect to their biogenetic relationship and similar ECD curve. In addition, the obtained ECD spectrum of **6** matched the calculated one of **6a**, suggesting a 23*S*-configuration for **6**. Therefore, **6** was assigned as 23*S*-hydroxy-2, 3-seco-tirucalla-8, 24-dien-2, 3, 21-trioic acid.

Comparing the spectroscopic data of **7**−**16** with the published ones, their structures were determined to be 3*α*, 24*R*, 25-trihydroxytirucalla-8-en-21-oic acid (**7**) [21], 3*α*-hydroxy-tirucalla-8, 24-dien-21-oic acid (**8**) [25], elemonic acid (**9**) [25], 3-oxotirucalla-7, 9(11), 24-trien-21-oic acid (**10**) [10], *α*-amyrin (**11**) [26], *β*-boswellic acid (**12**) [27], acetyl *β*-boswellic acid (**13**) [28], acetyl 11-keto-*β*-boswellic acid (**14**) [27], *α*-boswellic acid (**15**) [27], 3*α*-hydroxy-lup-20(29)-en-24-oic acid (**16**) [29], respectively.

Nitric oxide (NO) makes significant contribution to the physiology and pathology of many tissues including the immune system [30]. Through the Griess reaction [31], **1**−**16** were tested for the activities of reducing NO formation activated by LPS in murine microglial BV-2 cells, using 2-methyl-2-thiopseudourea sulfate (SMT) as a positive control. As shown in Table 3, the IC_{50} value of **9** was 7.58 ± 0.87 μmol·L^{-1}, which showed appreciable inhibitory activity (the IC_{50} value of SMT was 1.7 μmol·L^{-1}). The IC_{50} values of compounds **2**, **3**, **5**, and **8** were 24.0 ± 2.73, 20.0 ± 0.49, 46.7 ± 6.31, and 18.76 ± 0.64 μmol·L^{-1}, respectively, which also showed moderate inhibitory activities. The 2, 3-*seco*-ring A (**5**) of tirucallen-21-oic acid derivative decreased the activity. The cytotoxiticy of these compounds to BV-2 cells was measured by MTT assay. The results indicated that compounds **2**, **3**, **5**, **8**, and **9** produced no cytotoxic activities to BV-2 cells, while the possible biological effects caused by toxicity were excluded. On the other hand, the remaining compounds exhibited cell toxicity to vaious extent. Compound **10** exhibited cell toxicity at the concentration of 3 μmol·L^{-1}, compounds **1**, **6**, **7**, **12**, and **14** exhibited cell toxicity at the concentration of 10 μmol·L^{-1}, **4**, **11**, **13**, **15** exhibited cell toxicity at the concentration of 30 μmol·L^{-1} and **16** at 50 μmol·L^{-1}.

In conclusion, six new tirucallane-type triterpenoids were isolated from the CH_2Cl_2 extracts of the resins of *Boswellia carterii*. The calculated ECD method was employed to determine the absolute configurations of the new compounds (**1**, **2** and **5**). To our best knowledge, compounds **5** and **6** represented the first reported 2,3-*seco* tirucallane-type triterpenoids. In the *in vitro* NO inhibition experiment, some of the isolated compounds produced inhibitory effects on NO production in BV-2 cells induced by LPS, suggesting that tirucallane-type triterpenoids are the anti-inflammatory active constituents of olibanum.

Table 3 IC_{50} values of compounds 2, 3, 5, 8 and 9 for NO inhibitory activities in BV-2 cells (mean ± SD, *n* = 3)

Compound	IC_{50}/(μmol·L^{-1})	Compound	IC_{50}/(μmol·L^{-1})
2	24.00 ± 2.73	**3**	20.00 ± 0.49
5	46.70 ± 6.31	**8**	18.76 ± 0.64
9	7.58 ± 0.87	SMT [a]	1.70 ± 0.06

[a] SMT (2-methyl-2-thiopseudourea sulfate) was used as a positive control

Experimental

General experimental procedures

Optical rotation values were determined with a JASCO DIP-370 digital polarimeter (JASCO, Tokyo, Japan). HR-ESI-MS data were acquired using a Bruker microTOF-Q mass spectrometer. All NMR spectra were obtained on a Bruker ARX 400 NMR spectrometer and a Bruker AV 600 NMR spectrometer, using TMS as an internal reference standard. Semi-preparative HPLC was performed on a YMC ODS-A column (YMC Co., Ltd., Kyoto, Japan) consisting of a LC-6AB pump and a SPD-20AB UV detector (Shimadzu Co., Ltd., Kyoto, Japan). Silica gel (100−200 and 200−300 mesh, Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), Sephadex LH-20 (GE Healthcare, Uppsala, Sweden), and ODS (50 μm, YMC Co., Ltd., Kyoto, Japan) were used for column chromatography (CC). Precoated silica gel glass plate (GF_{254}, Qingdao Haiyang Chemical Co., Ltd., Qingdao, China) was used in TLC experiment.

Plant materials

The resin of *Boswellia carterii* was purchased from Beijing Tongrentang Drugstore (Shenyang, China) and authenticated by Professor LU Jin-Cai (Shenyang Pharmaceutical University, China). A voucher specimen (RX-20140409) was deposited in the Department of Natural Products Chemistry, Shenyang Pharmaceutical University, Shenyang, China.

Extraction and isolation

Briefly, 2200 g extract of the resin of *B. carterii* (4000 g) was obtained by refluxing extraction with CH_2Cl_2 three times, which was then separated through silica gel column chromatography (CC) with petroleum ether (PE)−EtOAc (100 : 0 to 80 : 20, *V/V*) as an eluent and gave five fractions (A−E). A large amount of four major constituents including acetyl *α*-boswellic acid, **11**, **13** and **14** were obtained by recrystallization from Frs. A−C. Fr. A (78.4 g) was chromatographically separated on a silica gel column using PE−EtOAc (100 : 0 to 0 : 100, *V/V*) system, obtaining six subfractions (A1−A6). Fr. A4 was fractionated by silica gel column with PE−EtOAc to afford 14 subfractions (A4-1 to A4-14). Compound **11** (4 mg) was obtained from Fr. A4-12 by repeated silica gel CC and recrystallization. Fr. A4-14 was applied to ODS CC with an elution of MeOH−H_2O (10% to 100%, *V/V*) to obtain **9** (2 g) and **13** (20 mg). Fr. B (38.7 g) was fractionated by eluting with PE−EtOAc (100 : 0 to 100 : 50, *V/V*) on a silica gel CC to afford eight subfractions (B1−B8). Fr. B7 was further separated to give five subfractions (B7-1 to B7-5), where Fr. B7-3 was recrystallized to afford **8** (4 mg). Moreover, Fr. B7-5 was further chromatographed to afford seven subfractions, in which Fr. B7-5-7 was subjected to pre-

parative TLC (PE−acetone, 3 : 1, *V/V*) to give a mixture, which was then separated by Sephadex LH-20 CC (MeOH) and semi-preparative HPLC with MeOH−H_2O (91 : 9, *V/V*, 2 mL·min^{-1}) to afford **12** (2 mg, t_R 102 min), **15** (2 mg, t_R 90 min), and **16** (2 mg, t_R 70 min). Fr. B8 was fractionated and recrystallized to obtain **10** (8 mg). Fr. C (43.4 g) was eluted with PE−EtOAc (100 : 0 to 100 : 100, *V/V*) to afford five subfractions (C1 to C5). Fr. C2 was recrystallized to give **14** (2.5 g). Fr. C4 was further eluted with PE−EtOAc (100 : 0 to 100 : 50, *V/V*) to obtain five subfractions (C4-1 to C4-5). Fr. C4-5 was applied to ODS CC using MeOH−H_2O (49% to 100%, *V/V*) to obtain seven fractions (C4-5-1 to C4-5-7). Fr. C4-5-6 was separated by Sephadex LH-20 CC (MeOH) and semi-preparative HPLC with MeOH−H_2O (72 : 28, *V/V*, 2 mL·min^{-1}) to afford **1** (4 mg, t_R 87 min), **3** (8 mg, t_R 112 min), and **4** (2 mg, t_R 106 min). Fr. C4-5-7 was separated by semi-preparative HPLC with MeOH−H_2O (75 : 25, *V/V*, 2 mL·min^{-1}) to afford **2** (10 mg, t_R 100 min). Fr. C5 was applied to ODS CC using MeOH–H_2O (55% to 100%, *V/V*) to afford 14 subfractions (C5-1 to C5-14). Fr. C5-9, Fr. C5-11, and Fr. C5-14 were recrystallized to give **5** (16 mg), **6** (2 mg), and **7** (10 mg), respectively.

Identification of new compounds

*3α, 11β-Dihydroxy-7-oxo-tirucalla-8, 24-dien-21-oic acid (**1**)*

Colorless needles; $[\alpha]_D^{20}$ −31.2 (*c* 0.14, MeOH); HR-ESI-MS *m/z* 485.3272 $[M - H]^-$ (Calcd. for $C_{30}H_{45}O_5$, 485.3272); ^{1}H and ^{13}C NMR (Tables 1 and 2); ECD (*c* 0.47 mg·mL^{-1}, MeOH) λ_{max} ($\Delta\varepsilon$) 344.5 (+11.06), 261.5 (−20.09), 235.5 (+4.80), 209.5 (−14.97) nm.

*11β-Hydroxy-3, 7-dioxo-tirucalla-8, 24-dien-21-oic acid (**2**)*

Colorless needles; $[\alpha]_D^{20}$ −50.4 (*c* 0.60, MeOH); HR-ESI-MS *m/z* 483.3102 $[M - H]^-$ (Calcd. for $C_{30}H_{43}O_5$, 483.3116); ^{1}H and ^{13}C NMR (Tables 1 and 2); ECD (*c* 0.65 mg·mL^{-1}, MeOH) λ_{max} ($\Delta\varepsilon$) 347.5 (+6.69), 262 (−15.03), 233.5 (+4.38) nm.

*24R, 25-Dihydroxy-3-oxo-tirucalla-8-en-21-oic acid (**3**)*

White amorphous powder; $[\alpha]_D^{20}$ −30.0 (*c* 0.11, MeOH); HR-ESIMS *m/z* 487.3406 $[M - H]^-$ (Calcd. for $C_{30}H_{47}O_5$, 487.3429); ^{1}H and ^{13}C NMR (Tables 1 and 2); ECD (*c* 0.42 mg·mL^{-1}, MeOH) λ_{max} ($\Delta\varepsilon$) 286.5 (+4.22), 211.5 (−10.71) nm.

*24R, 25-Dihydoxy-3-oxo-tirucalla-7, 9(11)-dien-21-oic acid (**4**)*

White amorphous powder; $[\alpha]_D^{20}$ −21.9 (*c* 0.12, MeOH); HR-ESIMS *m/z* 485.3269 $[M - H]^-$ (Calcd. for $C_{30}H_{45}O_5$, 485.3272); ^{1}H and ^{13}C NMR (Tables 1 and 2); ECD (*c* 0.46 mg·mL^{-1}, MeOH) λ_{max} ($\Delta\varepsilon$) 232 (−4.84) nm.

*2, 3-seco-Tirucalla-8, 24-dien-2, 3, 21-trioic acid (**5**)*

Colorless needles; $[\alpha]_D^{20}$ −9.2 (*c* 0.12, MeOH); HR-ESI-MS *m/z* 525.3193 $[M + Na]^+$ (Calcd. for $C_{30}H_{46}O_6Na$, 525.3187); ^{1}H and ^{13}C NMR (Tables 1 and 2); ECD (*c* 0.24 mg·mL^{-1}, MeOH) λ_{max} ($\Delta\varepsilon$) 228 (+11.72) nm.

*23S-Hydroxy-2, 3-seco-tirucalla-8, 24-dien-2, 3, 21-trioic acid (**6**)*

Colorless needles; $[\alpha]_D^{25}$ −14.5 (*c* 0.10, MeOH); HR-ESI-MS *m/z* 499.3060 $[M - H_2O - H]^-$ (Calcd. for $C_{30}H_{43}O_6$, 499.3065); ^{1}H and ^{13}C NMR (Tables 1 and 2); ECD (*c* 0.45 mg·mL^{-1}, MeOH) λ_{max} ($\Delta\varepsilon$) 225.5 (+14.67), 208.5 (−6.03), 199 (+3.64) nm.

Methylation of compound 3

A solution of **3** was made up by addition of 4 mg of **3** into 1 mL of anhydrous DMF. Then, anhydrous potassium carbonate (2.5 mg) was added under room temperature and stirred for 30 min. Then, MeI (5 μL) was added slowly, and the mixture was stirred under room temperature for 24 h, before poured into 20 mL of H_2O followed by extraction with 10 mL of EtOAc three times. The EtOAc layer was combined and washed with normal saline. After that, the liquid was dried using anhydrous Na_2SO_4 and concentrated in vacuo. Finally, **3a** (2.1 mg) was obtained by purifying the crude product with silica gel CC (PE−EtOAc 2 : 1, *V/V*).

*Determination of the absolute configuration of the 24, 25-diol moieties in compound **3***

The following experiment was performed according to previous studies [22-24]. Briefly, 0.5 mg·mL^{-1} of diol/$Mo_2(OAc)_4$ was subjected to ECD determination for **3a** in a ratio of 1 : 2. Then it was mixed and the first ECD spectrum was promptly recorded at room temperature, and then the inherent ECD was subtracted. At 300−340 nm, the diagnostic band was observed in the induced ECD spectrum, which was correlated to the absolute configuration of the 24, 25-diol moiety.

Computational methods

The Spartan 14.0 (Wavefunction Inc., Irvine, CA, USA) search with molecular mechanics MMFF was conducted for compounds **1**−**3**, **5**, and **6**. The meaningful conformers were further optimized using semi-empirical method in Gaussian 09 program. Those geometries were further optimized at the B3LYP/6-31+G (d, p) level of density functional theory (DFT), but no imaginary frequencies were observed. The conductor polarizable continuum model (CPCM) was used for solvent effects. The ECD of each conformer was calculated at B3LYP/6-31++G (d, p) level and they were summed up by Botlzmann averaging of all the conformers in SpecDis 1.51 [32]. The calculated ECD spectra of different conformers were simulated with a half bandwidth of 0.3−0.4 eV.

The inhibitory effects on LPS-activated NO formation

BV-2 cells were cultured with DMEM media supplemented with 100 units·mL^{-1} penicillin/streptomycin and 10% inactivated foetal bovine serum at 37 °C under a 5% CO_2 atmosphere. Then, the cells were incubated on 96-well culture plates before pretreatment for 24 h. The cell density was 5 × 10^4 cells per well. After that, with SMT as a positive control, the cells were incubated with or without the tested compounds with the presence and absence of 0.15 μg·mL^{-1} of LPS for 20 h. Then, 50 μL of 0.1% *N*-(1-naphtyl)ethylenediamine in H_2O was mixed with 50 μL of 1% sulfanilamide in 5% phosphoric acid at a ratio of 1 : 1 to obtain the Griess reagent, with which the supernatant of the cells was reacted. The concentration of nitrite which was used as a parameter of NO synthesis, was measured based on the absorbance of the

mixture in 96-well culture plates [31].

Supporting Information

The 1D and 2D NMR, HR-ESIMS, and ECD spectra of compounds **1**−**6** are available as Supporting Information, and can be requested by sending E-mails to the corresponding authors.

References

[1] Banno N, Akihisa T, Yasukawa K, *et al*. Anti-inflammatory activities of the triterpene acids from the resin of *Boswellia carteri* [J]. *J Ethnopharmacol*, 2006, **107**(2): 249-253.

[2] Safayhi H, Boden SE, Schweizer S, *et al*. Concentration-dependent potentiating and inhibitory effects of *Boswellia* extracts on 5-lipoxygenase product formation in stimulated PMNL [J]. *Planta Med*, 2000, **66**(2): 110-113.

[3] Fan AY, Lao L, Zhang RX, *et al*. Effects of an acetone extract of *Boswellia carterii* Birdw. (Burseraceae) gum resin on adjuvant-induced arthritis in Lewis rats [J]. *J Ethnopharmacol*, 2005, **101**(1-3): 104-109.

[4] Mahajan B, Taneja SC, Sethi VK, *et al*. Two triterpenoids from *Boswellia serrata* gum resin [J]. *Phytochemistry*, 1995, **39**(2): 453-455.

[5] Suhail MM, Wu WJ, Cao A, *et al*. *Boswellia sacra* essential oil induces tumor cell-specific apoptosis and suppresses tumor aggressiveness in cultured human breast cancer cells [J]. *BMC Complem and Altern M*, 2011, **11**: 129.

[6] Krieglstein CF, Anthoni C, Rijcken EJ, *et al*. Acetyl-11-keto-beta-boswellic acid, a constituent of a herbal medicine from *Boswellia serrata* resin, attenuates experimental ileitis [J]. *Int J Colorectal Dis*, 2001, **16**(2): 88-95.

[7] Hoernlein RF, Orlikowsky TH, Zehrer C, *et al*. Acetyl-11-keto-*β*-boswellic acid induces apoptosis in HL-60 and CCRF-CEM cells and inhibits topoisomerase [J]. *J Pharmacol Exp Ther*, 1999, **288**(2): 613-619.

[8] Shao Y, Ho CT, Chin CK, *et al*. Inhibitory activity of boswellic acids from *Boswellia serrata* against human leukemia HL-60 cells in culture [J]. *Planta Med*, 1998, **64**(4): 328-331.

[9] Ammon H, Safayhi H, Mack T, *et al*. Mechanism of antiinflammatory actions of curcumine and boswellic acids [J]. *J Ethnopharmacol*, 1993, **38**(2-3): 105-112.

[10] Wang F, Li ZL, Cui HH, *et al*. Two new triterpenoids from the resin of *Boswellia carterii* [J]. *J Asian Nat Prod Res*, 2011, **13**(3): 193-197.

[11] Cui R, Zhou JY. Progress on the chemical and pharmacological research of frankincense [J]. *Chin Pharm J*, 2003, **38**(6): 407-409.

[12] Yoshikawa M, Morikawa T, Oominami H, *et al*. Absolute stereostructures of olibanumols A, B, C, H, I, and J from olibanum, gum-resin of *Boswellia carterii*, and inhibitors of nitric oxide production in lipopolysaccharide-activated mouse peritoneal macrophages [J]. *Chem Pharm Bull*, 2009, **57**(9): 957-964.

[13] Morikawa T, Oominami H, Matsuda H, *et al*. Four new ursane-type triterpenes, olibanumols K, L, M, and N, from traditional Egyptian medicine olibanum, the gum-resin of *Boswellia carterii* [J]. *Chem Pharm Bull*, 2010, **42**(13): 1541-1544.

[14] Morikawa T, Oominami H, Matsuda H, *et al*. New terpenoids, olibanumols D–G, from traditional Egyptian medicine olibanum, the gum-resin of *Boswellia carterii* [J]. *J Nat Med*, 2011, **65**(1): 129-134.

[15] Corsano S, Nicoletti R. The structure of incensole [J]. *Tetrahedron*, 1967, **23**(4): 1977-1984.

[16] Nicoletti R, Forcellese ML. The structure of isoincensole-oxide [J]. *Tetrahedron*, 1968, **24**(22): 6519-6525.

[17] Li FS, Xu KP, Yuan SH, *et al*. Macrocyclic diterpenes from *Boswellia carterii* Birdwood (Frankincense) [J]. *Chin J Org Chem*, 2010, **30**(1): 107-111.

[18] Wang YG, Ren J, Wang AG, *et al*. Hepatoprotective prenylaromadendrane-type diterpenes from the gum resin of *Boswellia carterii* [J]. *J Nat Prod*, 2013, **76**(11): 2074-2079.

[19] Liu ZG, Li ZL, Bai J, *et al*. Anti-inflammatory diterpenoids from the roots of *Euphorbia ebracteolata* [J]. *J Nat Prod*, 2014, **77**(4): 792-799.

[20] Hu P, Li DH, Wang KB, *et al*. New phenolic compounds from *Vitex negundo* var. *heterophylla* and their antioxidant and NO inhibitory activities [J]. *J Func Foods*, 2015, **19**: 174-181.

[21] Liu YH, Abreu P. Tirucallane triterpenes from the roots of *Ozoroa insignis* [J]. *Phytochemistry*, 2006, **67**(13): 1309-1315.

[22] Liu J, Liu YB, Si YK, *et al*. New vernocuminosides from the stem barks of *Vernonia cumingiana* Benth [J]. *Steroids*, 2009, **74**(1): 51-61.

[23] Górecki M, Jabłońska E, Kruszewska A, *et al*. Practical method for the absolute configuration assignment of tert/tert 1, 2-diols using their complexes with $Mo_2(OAc)_4$ [J]. *J Org Chem*, 2007, **72**(8): 2906-2916.

[24] Frelek J, Klimek A, Ruskowska P. Dinuclear transition metal complexes as auxiliary chromophores in chiroptical studies on bioactive compounds [J]. *Curr Org Chem*, 2003, **7**(11): 1081-1104.

[25] Marques DD, Graebner IB, de Lemos TL, *et al*. Triterpenes from *Protium hebetatum* resin [J]. *Nat Prod Commun*, 2010, **5**(8): 1181-1182.

[26] Ganeva Y, Tsanlcova E, Simova S, *et al*. Rofficerone: a new triterpenoid from *Rosmarinus officinalis* [J]. *Planta Med*, 1993, **59**(3): 276-277.

[27] Zhou JY, Cui R. Chemical components of *Boswellia carterii* [J]. *Acta Pharm Sin*, 2002, **37**(8): 633-635.

[28] Belsner K, Büchele B, Werz U, *et al*. Structural analysis of pentacyclic triterpenes from the gum resin of *Boswellia serrata* by NMR spectroscopy [J]. *Magn Reson Chem*, 2003, **41**(2): 115-122.

[29] Culioli G, Mathe C, Archier P, *et al*. A lupane triterpene from frankincense (*Boswellia* sp., Burseraceae) [J]. *Phytochemistry*, 2003, **62**(4): 537-541.

[30] Karpuzoglu E, Ahmed SA. Estrogen regulation of nitric oxide and inducible nitric oxide synthase (iNOS) in immune cells: Implications for immunity, autoimmune diseases, and apoptosis [J]. *Nitric Oxide*, 2006, **15**(3): 177-186.

[31] Wang MC, Zhang Q, Wang H, *et al*. Characterization and NO inhibitory activities of chemical constituents from an edible plant *Petasites tatewakianus* [J]. *J Agr Food Chem*, 2014, **62**(38): 9362-9367.

[32] Bruhn T, Schaumlöffel A, Hemberger Y, *et al*. Quantifying the comparison of calculated and experimental electronic circular dichroism spectra [J]. *Chirality*, 2013, **25**(4): 243-249.

Cite this article as: LIU Fang-Shen, ZHANG Ting-Ting, XU Jun, JING Qin-Xue, GONG Chi, DONG Bang-Jian, LI Da-Hong, LIU Xiao-Qiu, LI Zhan-Lin, YUAN Zhong, HUA Hui-Ming. New tirucallane-type triterpenoids from the resin of *Boswellia carterii* and their NO inhibitory activities [J]. *Chin J Nat Med*, 2021, **19**(9): 686-692.