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One Novel Nortriterpenoid from the Mastic (*Pistacia lentiscus*) and Its Anti-Inflammatory Activity

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ABSTRACT

A novel pair of oleanane nor-triterpenes, with compound **1** featuring a unique 18 α -H structure, was isolated from mastic, and this compound represents a noteworthy new entity not previously reported in the literature. The absolute configurations of their structures were further determined using a combination of different analytical methods such as NMR, high-resolution mass spectrometry (HR-MS), ultraviolet (UV), infrared (IR) and single-crystal X-ray diffraction (SXRD). The compound actively mitigated inflammations by efficiently quenching nitric oxide (NO) synthesis within an *ex vivo* system using lipopolysaccharide activated murine macrophage RAW264.7 cells. Moreover, compound **1** exhibit a better IC₅₀ concentration of 11.69 $\pm$ 1.39 μ M, surpassing the efficacy of the positive control dexamethasone, which exhibited an IC₅₀ of 23.21 $\pm$ 1.17 μ M. While compound **2** also demonstrated inhibitory activity, its potency was comparatively weaker, with an IC₅₀ of 26.18 $\pm$ 2.66 μ M.

KEYWORDS

Mastic; nor-triterpene; 18 α -H oleanane; anti-inflammatory

1 Introduction

Pentacyclic triterpenoids, owing to their remarkable pharmacological activities, have emerged as promising structural types for new drug candidates, capturing the attention of researchers worldwide [1,2]. Pentacyclic triterpenoids are found to exert anti-inflammatory influences in the majority of studies. Martín and colleagues have established that oleanolic acid has significant anti-Th1 cell-mediated inflammatory diseases [3]. The following study also showed that glycyrrhizic acid inhibits CVB-3-induced myocarditis by down-regulating tumor necrosis factors [4]. Saikosaponin exhibits strong anti-inflammatory potential, and it could decrease neuroinflammation and its associated neurodegeneration by inhibiting nuclear factor- κ B (NF- κ B) mediated downstream signaling cascades *in vivo* [5]. It is observed that lupeol exhibited anti-neuroinflammatory properties in lipopolysaccharide (LPS)-induced microglial neuroinflammation through mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK)



pathways and has the potential to treat various neuroinflammatory diseases [6]. To date, four triterpenes have been approved for clinical use in humans [7]. However, further research is limited not only by expensive natural product sources but also by the complex procedures of chemical extraction and synthesis [7].

Mastic is a naturally occurring resin isolated from the stem or twig cuts of *Pistacia lentiscus* L. (Family Anacardiaceae), abundantly found in Southern regions of Chios, Greece [8,9]. Mastic is reported to have great applications to cure gastrointestinal disorders including indigestion, abdominal pain, and ulcer craters in classical Greek medicine. It was also used as a spice in foods, beverages, and cosmetics preparation [10–12]. Since the 1980s, from the literature on mastic, about 200 chemical compounds have been found including monoterpenes, sesquiterpenes, and triterpenes; and current research on its extracts has revealed that it possesses considerable anti-inflammatory, anticancer, and antioxidant [13,14]. Further chemical study of mastic for the anti-inflammatory activity and bioactive constituents [15,16], this research has led to the identification and characterization of a novel 18 α -H nor-triterpenoid compound, designated as (**1**), its diastereoisomer, the known 18 β -H nor-triterpenoid (**2**). Therefore, the isolation, structural identification, plausible biogenetic pathways, and discussion encompass the inhibitory impacts of the isolated compounds on NO synthesis in LPS-induced RAW 264.7 cells.

2 Results and Discussion

2.1 Structural Elucidation

The compound **1** was in the form of colorless needle shaped crystals having the molecular formula of C₂₉H₄₆O₂, was confirmed HR-ESI-MS analysis. This exhibited an ion peak at m/z 427.3585 [M + H]⁺ (calcd for C₂₉H₄₇O₂, 427.6576) (Fig. S1), supported by the data obtained from ¹³C nuclear magnetic resonance (NMR) spectroscopy, with 7 degrees of unsaturation. The diagnostic 1D NMR data (Figs. S2 and S3) implied the presence of an olefin signal (δ_H 5.38, 1H, t, J = 1.9 Hz; δ_C 120.8, 141.1), a carbonyl signal (δ_C 218.1), an oxygenated carbon signal (δ_C 70.8) and 7 methyls (δ_H 1.15, 1.10, 1.06, 1.05, 1.01, 0.97, 0.90, each 3H, all s). The information presented indicates that compound **1** is an oleanane nor-triterpenoid.

The plane structure of compound **1** was elucidated by interpreting its 2D NMR data, which included HSQC, ¹H-¹H COSY, and HMBC analyses (Figs. S4–6). **1** was assigned to 7 methyls, 10 methylenes, 4 methines (together with one olefin carbon) and 8 quaternary carbons (including one carbonyl carbon, one oxygen-containing carbon and one olefin carbon) by HSQC spectrum. Fig. 1 illustrates that the ¹H-¹H COSY spectrum disclosed the existence of six spin-spin coupling segments. The HMBC correlations delineated the connectivity as follows from H₃-23/H₃-24 to C-3 (δ_C 218.1), C-4 (δ_C 47.5), and C-5 (δ_C 55.3); from H₃-25 to C-1 (δ_C 39.7), C-5, C-9 (δ_C 46.6) and C-10 (δ_C 36.7); from H₃-26 to C-7 (δ_C 33.7), C-8 (δ_C 39.6), C-9 and C-14 (δ_C 43.7); from H₃-27 to C-8, C-13 (δ_C 141.1), C-14 and C-15 (δ_C 26.1); from H-12 to C-9, C-14 and C-18 (δ_C 40.3); from H₃-29/H₃-30 to C-19 (δ_C 37.6), C-20 (δ_C 30.9) and C-21 (δ_C 33.8); from H₂-15, H₂-16, H₂-19, H₂-21 and H₂-22 to C-17 (δ_C 70.8), **1** was established as an oleanane nor-triterpenoid, and its plane configuration was depicted.

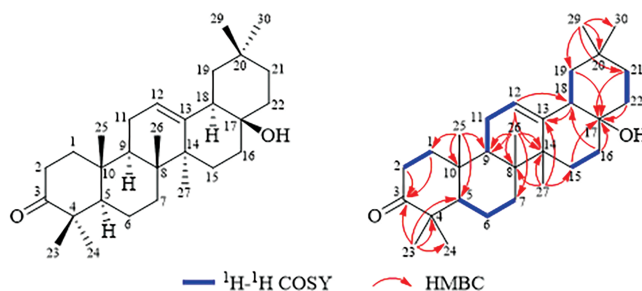


Figure 1: Structure and key COSY, HMBC correlations of compound **1**

Compound **1** has the same planar structure as 3-oxoolean-12-en-28-ol [17], known as compound **2** isolated from mastic. The NMR data concerning the region C-16 to C-19 seems contradictory, and it has been identified that additional knowledge regarding the relative configuration of compound **1** is needed, which requires further study to determine the relative configuration of **1**. NOESY (Fig. S7) correlations depicted in Fig. 2, which connect H₃-27 to H-9 and H-18, H-18 to H₃-30, and H-5 to H₃-24, suggest that these protons are positioned co-facial to each other and are designated with an α -orientation. Based on these findings, the alignment of H-18 is confirmed to be in the α -configuration for compound **1**, contradicting the typical configuration observed in oleanane triterpenes. In addition, the orientation of the 17-OH could not be determined through 2D NMR correlation data.

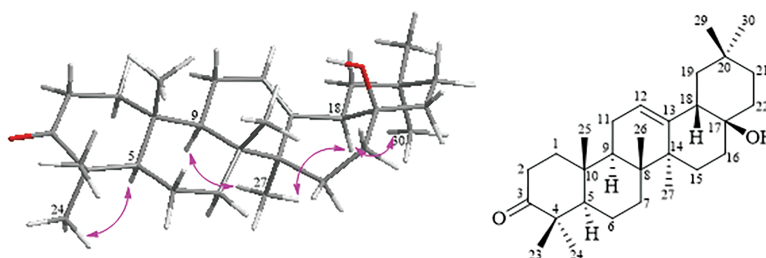


Figure 2: Key NOESY correlations for compound **1** (left) and structural for compound **2** (right)

We were fortunate to acquire single crystals for both compounds **1** and **2** from a methanol solution, which allowed us to definitively determine their absolute configurations via X-ray single-crystal diffraction studies (Fig. 3). Ultimately, the crystallographic analysis via X-ray diffraction of compound **1** disclosed its enantiomeric configuration as 5*R*, 8*R*, 9*R*, 10*R*, 14*S*, 17*S*, 18*R*, accompanied by a Flack parameter of 0.03(16). Similarly, the β -orientation of both H-18 and OH-17 in **2** was elucidated.

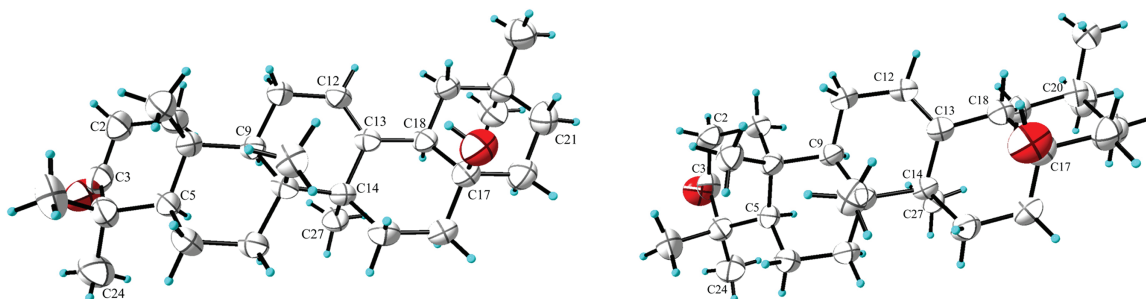
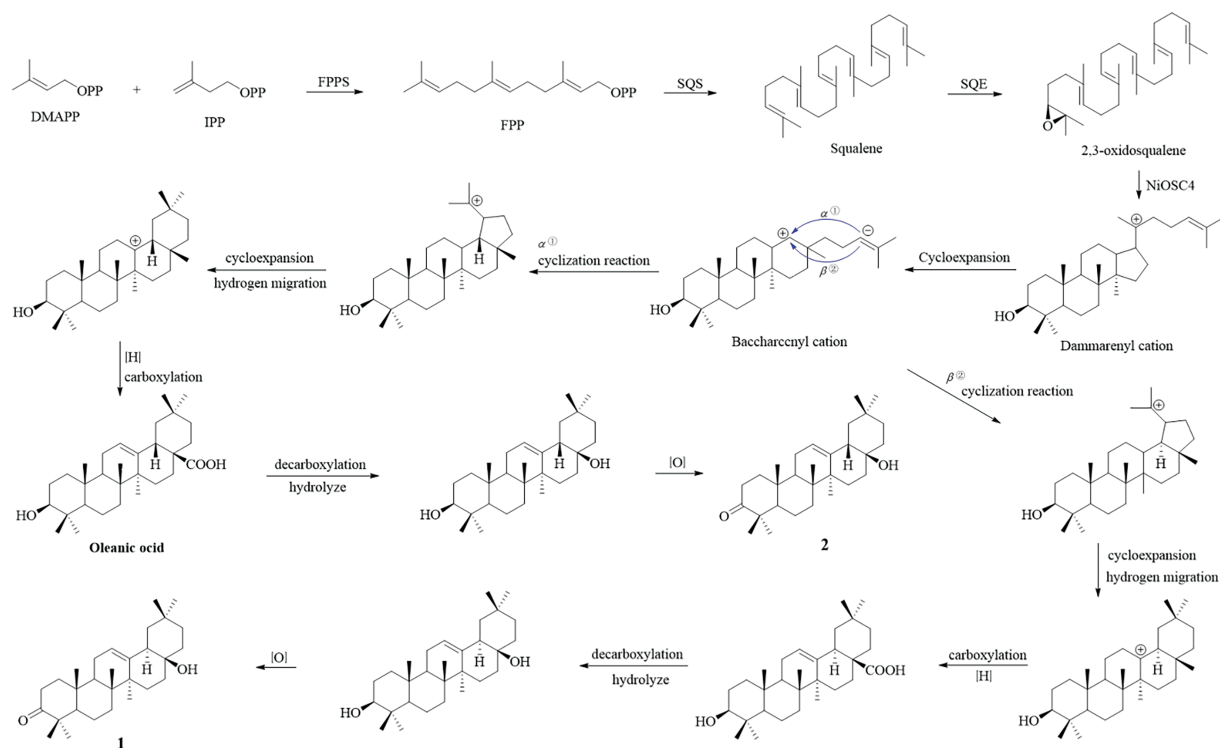


Figure 3: Single crystal diffraction conformation of compounds **1** (left) and **2** (right)

2.2 Elucidation of Potential Biosynthetic Pathways

Within the pentacyclic triterpenes, the α -orientation of H-18 is quite uncommon, with only the 18 α and 18 β isomers of glycyrrhizinic acid having been identified in *Glycyrrhiza glabra* [18]. A speculative metabolic pathway for the synthesis of compounds **1** and **2**, starting from the assumed squalene precursor, has been delineated in Scheme 1. Initially, squalene synthase catalyzes the dimerization of farnesyl diphosphate (FPP) to yield squalene, which is subsequently converted into 2,3-oxidosqualene through the action of squalene epoxidase (SQE) [19]. In the presence of the *NiOSC4* enzyme, 2,3-oxidosqualene undergoes a cyclization reaction to yield the dammarenyl cation [20]. The dammarenyl cation subsequently experiences ring expansion, yielding the 6,6,6,6-baccharocnyl cation in this scenario, π electrons can assail the carbonium ion from either direction [21,22], culminating in the generation of

intermediates with distinct H-18 orientations. Subsequently, a set of chiral isomers with distinct 18-H orientations is produced through a sequence of reactions that involve ring expansion, carboxylation, decarboxylation, hydrolysis and oxidation.



Scheme 1: Plausible biosynthetic pathways for compounds **1** and **2**

2.3 Anti-Inflammatory Activity Assay

The subsequent cytotoxicity assay data demonstrated that at a concentration of 80 μM , compounds **1** and **2** yields survival rate of 68.6% and 79.0% in RAW 264.7 cells, respectively, which is similar to the positive control dexamethasone and hence rules out the chance of a false positive results in the inhibition of NO formation. Compound **1** displayed marked anti-inflammatory properties, specifically in the inhibition of NO generation in the RAW264.7 cells, showed an IC_{50} value of $11.69 \pm 1.39 \mu\text{M}$, pared with the positive control dexamethasone ($\text{IC}_{50} = 23.21 \pm 1.17 \mu\text{M}$) and compound **2** ($\text{IC}_{50} = 26.18 \pm 2.66 \mu\text{M}$). The obtained results correlate with the literature data that describe compounds possessing the H-18 α -orientation structural characteristic as more active than those with β -H orientation [23,24].

3 Experimental Section

3.1 Routine Experimental Techniques

Optical rotations were obtained through a digital Autopol-IV polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA). Infrared (IR) spectral data was obtained with a Cary 630 spectrometer manufactured (Agilent Technology). The nuclear magnetic resonance (NMR) (Bruker AV-400, Berlin, Germany) spectra were obtained by using TMS standards. X-single-crystal diffraction data were obtained by using XtalABoxford diffraction (Tokyo, Rigaku, Japan). Semi preparative HPLC analyses were done through Hitachi Primaide apparatus equipped with 1110 pump interfaced with a 1430 diode array detector, 1210 autosampler with RP-C18 columns (5 μm , 10 mm $\times$ 250 mm dimensions, YMC-pack ODS-A). Q-Exactive tandem mass spectrometer was used to obtain ESI-HR-MS data (Thermo Fisher,

Waltham, MA, USA). The optical density (OD) was measured by Multiskan GO full wavelength enzyme labeler (Thermo Fisher). Silica gel (300–400 mesh, Qingdao Marine Chemical Inc., Qingdao, China), MCI gel (GHP20/P120, Mitsubishi Chemicals, Tokyo, Japan), and YMC ODS-A (YMC, Kyoto, Japan) gel was successfully employed during column chromatography. High performance liquid chromatography (HPLC) grade higher purity solvents (Sigma-Aldrich, Saint Louis, MO, USA), and analytical-grade solvents were obtained from the local supplier (Tansooole, Shanghai, China).

3.2 Plant Materials

Samples of commercial mastic gum used in this study were purchased from Xinjiang Ensar Uyghur Medicine Pieces Co., Ltd. (Hetian, China) on 16 March 2022, and properly authenticated from Xiao-rong Yang, School of Biological Sciences and Technology, Yili Normal University. A specific voucher specimen number (HX-MG-220316) issued and lodged within the Key Lab of Clean Conversion and HighValue Utilization of Biomass Resources, Yili Normal University.

3.3 Extraction and Isolation Procedure

A mass of mastic, precisely 500 g, underwent extraction with a MeOH:EA mixture (3:1 ratio) (total volume 800 mL), employing the maceration method at ambient temperature for a duration of approximately 72 h, yielding 349.6 g of a crude extract. A segment of the initial extract, specifically 337.2 g, was thereafter directed through normal-phase chromatography on a silica gel matrix, utilizing a petroleum ether:ethyl acetate (PE:EA) gradient system established in the ratio from (100:1) to (0:1) was used as the eluent. By employing this procedure, twelve fractions (Fr.1–Fr.12) were obtained from the extract. Fr.2 (13.3 g) was employed to MCI column chromatography (MeOH-H₂O, 70:30–100:0) with gradient elution to give five sub-fractions (Fr.2A–Fr.2E). Fr. 2B (449.4 mg) was employed on a silica gel column eluting with a gradient of DCM-MeOH (180:1–0:1) to give seven sub-fractions (Fr. 2B1–Fr. 2B7), Fr. 2B3 (252.0 mg) was run on a silica gel column eluting with a gradient of PE-EA (20:1–0:1) to give four sub-fractions (Fr. 2B3a–Fr. 2B3d), Fr. 2B3d (28.4 mg) was purged by semi preparative HPLC analysis (85% ACN-H₂O, 3 mL/min) to give compound **2** (16.7 mg, t_R = 37.1 min). Fr. 2C (597.8 mg) was chromatographed on a silica gel column eluting with a gradient of PE-EA (30:1–0:1) to give eight sub-fractions (Fr. 2C1–Fr. 2C8), Fr. 2C6 (262.2 mg) was subjected to ODS column chromatography (MeOH-H₂O, 70:30–100:0) with gradient elution to give five subfractions (Fr. 2C6a–Fr. 2C6e). Fr. 2C6e (37.0 mg) was chromatographed on a silica gel column eluting with a gradient of PE-EA (20:1–0:1) to give two sub-fractions (Fr. 2C6e1–Fr. 2C6e2), Fr. 2C6e1 (17.5 mg) was purified by semi preparative HPLC (87% MeOH-H₂O, 3 mL/min) to give compound **1** (9.4 mg, t_R = 29.3 min).

18 α -H-3-oxoolean-12-en-28-ol (**1**): colorless crystals (methanol), $[\alpha]_D^{25} + 66^\circ$ (c 0.300, MeOH); UV (MeOH) λ_{max} 205.2 nm; IR ν_{max} 2933, 2856, 1704, 1541, 1385, 965 cm^{−1} (Fig. S8); ¹H NMR (CDCl₃, 400 MHz) and ¹³C NMR (CDCl₃, 100 MHz) (Table 1); HR-ESI-MS m/z 427.3585 [M + H]⁺ (calculated for C₂₉H₄₇O₂, 427.3576).

3.4 X-Ray Crystallographic Analysis

After undergoing recrystallization in methanol at room temperature, compounds **1** and **2** resulted in colorless crystalline forms. X-ray diffraction data were obtained an XtalABoxford diffractometer aligned with Cu K α radiation (λ = 1.54178 Å). The crystalline structures were determined employing the ShelXT program through intrinsic phasing and refined via the ShelXL package, employing least-squares minimization. These crystalline structures were elucidated by utilizing the ShelXT software for structure solution via intrinsic phasing, followed by refinement with the ShelXL program employing least-squares minimization techniques. The corresponding crystallographic information has been archived at the CCDC under reference numbers 2,386,881 for compound **1** (Fig. S9) and 2,386,883 for compound **2** (Fig. S10).

Crystal data for compound **1**. $2(\text{C}_{29}\text{H}_{46}\text{O}_2)$, $M = 853.31$ Da, orthorhombic, space group $P2_12_12_1$, $a = 7.43070$ (10) Å, $b = 19.8885$ (4) Å, $c = 34.0537$ (8) Å, $\alpha = 90^\circ$, $\beta = 90^\circ$, $\gamma = 90^\circ$, $V = 5032.64$ (17) Å³, $T = 293$ (2) K, $Z = 4$, Cu $K\alpha$ Radiation ($\lambda = 1.54184$), $F(000) = 1888$, $\rho_{\text{calc}} = 1.126$. A total of 12,856 reflections were noted in the range of $5.07^\circ \leq 2\theta \leq 133.668^\circ$. The final R_1 value was 0.0538, and the ωR_2 value was 0.1510 (all data). The goodness of fit on F2 was 1.030. Flack parameter = -0.03 (16) (Table S1).

Table 1: ^1H and ^{13}C NMR spectral data of compound **1** (J in Hz, δ in ppm)

No.	δ_{H}	δ_{C}	No.	δ_{H}	δ_{C}
1	1.93 overlap, 1.44 m	39.7, CH ₂	16	1.54 overlap, 1.58 overlap	35.8, CH ₂
2	2.51, 2.42 m	34.3, CH ₂	17	–	70.8, C
3	–	218.1, C	18	2.28 m	40.3, CH
4	–	47.5, C	19	1.25 m	37.6, CH ₂
5	1.36 m	55.3, CH	20	–	30.9, C
6	1.48 m	19.8, CH ₂	21	1.51 overlap, 1.15 overlap	33.8, CH ₂
7	1.57 overlap	33.7, CH ₂	22	1.51 overlap	35.2, CH ₂
8	–	39.6, C	23	1.05 s	21.6, CH ₃
9	1.49 overlap	46.6, CH	24	1.10 s	27.0, CH ₃
10	–	36.7, C	25	1.06 s	15.9, CH ₃
11	1.98 overlap, 1.91 overlap	23.8, CH ₂	26	1.01 s	18.0, CH ₃
12	5.38 t (1.90)	120.8, CH	27	1.15 s	22.5, CH ₃
13	–	141.1, C	29	0.90 s	24.5, CH ₃
14	–	43.7, C	30	0.97 s	33.6, CH ₃
15	2.00 overlap, 1.15 overlap	26.1, CH ₂			

Crystal data for compound **2**. $\text{C}_{29}\text{H}_{46}\text{O}_2$, $M = 426.66$ Da, orthorhombic, space group $P2_12_12_1$, $a = 10.84822$ (17) Å, $b = 14.5548$ (3) Å, $c = 15.6281$ (3) Å, $\alpha = 90^\circ$, $\beta = 90^\circ$, $\gamma = 90^\circ$, $V = 2467.58$ (8) Å³, $T = 293$ (2) K, $Z = 4$, Cu $K\alpha$ Radiation ($\lambda = 1.54184$), $F(000) = 944$, $\rho_{\text{calc}} = 1.148$. A total of 10,273 reflections were measured in the range of $5.658^\circ \leq 2\theta \leq 136.244^\circ$. The final R_1 value was 0.0412, and the ωR_2 value was 0.1277 (all data). The goodness of fit on F2 was 1.097. Flack parameter = -0.07 (12) (Table S1).

3.5 Cell Culture and Cell Viability Analysis

The Murine RAW 264.7 macrophage cells were cultured and maintained using Dulbecco's Modified Eagle's Medium (DMEM) contains FBS (10%) and penicillin/streptomycin (1%), in humidified incubator set at 37°C and 5% CO_2 . Cell stimulation was done using LPS ($2.0 \mu\text{g/mL}$). Prior to this, dimethylsulfoxide (DMSO) was used for initial solubilization of the compounds, and later further dilutions were prepared in supplemented DMEM.

Following certain modifications in an earlier reported method [25], RAW 264.7 macrophages were seeded (2.0×10^4) in each well using 96-wells plates, and allowed for incubation in fully supplemented DMEM for 24 h. Later on, DMEM was removed, and $100 \mu\text{L}$ of fresh non-supplemented DMEM mixed with compounds **1** and **2** was poured in each well and allowed for further incubation for 24 h period.

Following the guidelines for the CCK-8 assay, the medium was then replaced with 100 μL of CCK-8 reagents + fresh DMEM (1:9 dilution), and covered using aluminum foil. The cells were allowed for 2 h incubation in the dark, the absorbance of the plate was quantified at a wavelength of 450 nm.

3.6 Inhibition of NO Generation

The estimation of the total generation of reactive nitrogen species specifically nitrous oxide (NO) was done using Griess reagent following manufacturer's instructions (Beyotime, Shanghai, China) [26]. Approximately 2.0×10^4 RAW264.7 cells were seeded per well of the 96-well plate, and let them incubate for about 24 h, and then culture medium was replaced with the drug (0, 10, 20, 40, and 80 μM) and positive control media and allowed to incubate for additional 2 h. Next to it, recombinant LPS (2.0 $\mu\text{g/mL}$) was added into the cultured cells and further incubated for 24 h. For nitrite accumulation, an equal volume of Griess-I and -II reagents was combined with 50 μL aliquots of the supernatant from the media for final reaction. Optical density (OD) of this solution was measured at 540 nm. Dexamethasone served as the benchmark control in the experiment. The IC_{50} values were subsequently calculated using version 8.0 of the GraphPad Prism software.

4 Conclusion

In conclusion, a new nor-triterpene with a $18\alpha(\text{H})$ stereochemistry has been obtained from the extract of mastic for the first time. The pair of oleanane nor-triterpenes from the perspective of biotransformation may be generated through an enzyme-catalyzed series of reactions involving cyclization, oxidation, and decarboxylation starting from a squalene precursor. Anti-inflammatory assays indicated that compound **1** exhibited stronger inhibitory NO production activities (IC_{50} values $11.69 \pm 1.39 \mu\text{M}$) than that of **2** ($\text{IC}_{50} = 26.18 \pm 2.66 \mu\text{M}$). Thus, the above data demonstrate the essential contribution of the H-18 orientation to the variability of the structure-activity relationship. The present investigation provides significant information concerning the pharmacophoric features of triterpene derivatives that can be considered as new lead compounds for anti-inflammatory agents.

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Author Contributions: Yan Wu conducted bioactivity testing and authored the initial draft; Xuerui An performed isolations, confirmation and structural explanation of the isolated components; Haofan Lv and Zhiqiang Zhao managed the cultivation and analysis of single crystals; Wei Liu and Chunpeng Wan oversaw the experimental design and manuscript revision. All authors reviewed the results and approved the final version of the manuscript.

Availability of Data and Materials: The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest to report regarding the present study.

Supplementary Materials: The supplementary material is available online at <https://doi.org/10.32604/phyton.2025.059581>.

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