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Pepluenone, a new pepluane-type diterpenoid with inhibitory activity on the release of NO from the whole plant of *Euphorbia peplus* L.

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ABSTRACT

One new pepluane-type diterpenoid, pepluenone (**1**), and four known compounds were isolated from the whole plant of *Euphorbia peplus* L. Compound **1** possesses a 5/6/5/6 tetracyclic skeleton with a unique aromatic ring D. Its structure was determined based on spectroscopic data and X-ray crystallography. The biogenetic pathway of **1** was proposed, in which chemical transformation verified the aromatization step. *In vitro* anti-inflammatory activity tests indicated that Compounds **1** and **3** had a strong inhibitory effect on releasing NO from mouse macrophage RAW264.7 induced by LPS.

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Introduction

Euphorbia peplus L is an annual herb native to the Mediterranean coast and was later introduced to Yunnan, Guangzhou, and other places in China [1]. The plant has been used as a folk medicinal herb for treating skin diseases, asthma, and cancer [2–4]. *E. peplus* L is a rich source of macrocyclic diterpenoids, which exhibit a wide range of excellent biological activities, including anti-inflammatory, antitumor, antiviral, insecticidal, multidrug resistance, and other biological activities [5–10], which have attracted much attention from chemists and biologists [11–23].

Pepluane-type diterpenoids with a 5/6/5/6 tetracyclic skeleton showed significant anti-inflammatory activity. Nitric oxide (NO) is critical in regulating vasodilation and protecting vascular endothelial cells under normal physiological conditions [24]. However, excessive NO induces inflammation-related cell, tissue, and organ injury [25]. To identify more natural products owing to anti-inflammatory activity with NO inhibition, one undescribed compound, pepluenone (**1**), with a rare aromatic ring D, and four known compounds (**2**–**5**) were obtained from the whole plant of *E. peplus* L. (Fig. 1). The structure of the new compound has been elucidated by extensive NMR studies and further confirmed by X-ray crystal analysis. Then, chemical conversion helped us establish the aromatic moiety's biogenesis. We also performed anti-inflammatory activity tests on these compounds *in vitro*. Among them, Compounds **1** and **3** strongly inhibited NO release from mouse macrophage RAW264.7 cells induced by LPS.

Results and discussion

Pepluenone (**1**) was isolated as colourless crystals. Its molecular formula $C_{31}H_{36}O_8$, was determined by HRESIMS (*m/z* 559.2302 [M+Na]$^+$; calcd for $C_{31}H_{36}O_8Na$, 559.2305), with 14 degrees of unsaturation. The IR spectrum showed absorption bands assignable to hydroxyl (3495 and 3436 cm^{-1}) and aromatic ring (1622 and 1599 cm^{-1}) functionalities. The ^{1}H and ^{13}C NMR data (Table 1) of

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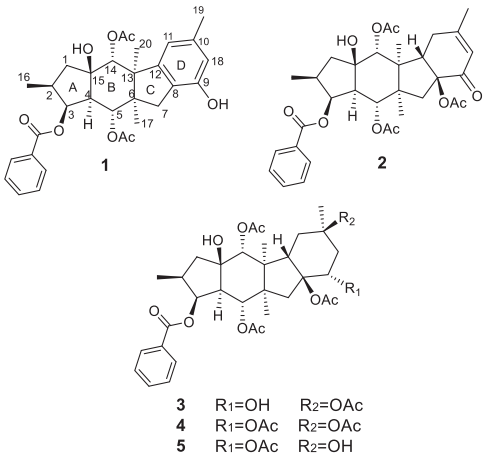


Fig. 1. Structures of Compounds 1–5.

Table 1
^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data for 1 in $CDCl_3$.

NO.	δ_H	δ_C
1a	1.55, dd (14.2, 5.1)	43.3 CH_2
1b	2.13, m	
2	2.41, dd (14.2, 6.3)	36.6 CH
3	5.72, t (6.3)	75.7 CH
4	2.45, dd (12.2, 4.8)	47.4 CH
5	5.28, d (12.2)	70.8 CH
6	–	51.7 C
7a	2.28 (d, 15.6)	37.2 CH_2
7b	2.65 (d, 15.6)	
8	–	121.9 C
9	–	153.0 C
10	–	138.1 C
11	6.67, s	115.6 CH
12	–	150.2 C
13	–	54.7 C
14	5.64, s	74.4 CH
15	–	83.5 C
16	0.95, br s	16.0 CH_3
17	1.26, s	15.5 CH_3
18	6.13, s	115.0 CH
19	2.19, s	21.5 CH_3
20	0.98, s	23.6 CH_3
15-OH	2.24, s	–
5-OAc	–	171.4 C
	2.01, s	21.2 CH_3
14-OAc	–	170.0 C
	2.18, s	21.0 CH_3
3-OBz	–	166.5 C
1′	–	129.8 C
2′,6′	7.20, d (8.1)	129.5 CH
3′,5′	7.81, m	128.2 CH
4′	7.36, t (7.4)	132.8 CH

1 showed resonances for two acetoxy groups (δ_H 2.01, 2.18; δ_C 171.4, 170.0) and one benzoyloxy group [δ_H 7.81 (2H, d, J = 8.3 Hz), 7.36 (1H, t, J = 7.4 Hz) and 7.20 (2H, t, J = 8.1 Hz); (δ_C 166.5, 129.8, 129.5 × 2, 128.2 × 2, 132.8)]. The remaining resonances include four methyls, two sp^3 methylenes, five sp^3 methines (three oxygenated at δ_C 70.8, 74.4, and 75.7), three sp^3 nonprotonated carbons (one oxygenated at δ_C 83.5) and six olefinic carbons, including two protonated (δ_C 115.0 and 115.6) and one oxygenated (δ_C 153.0). Apart from ten degrees of unsaturation occupied by three double bonds, two acetoxy groups and one benzoyloxy group, we assumed the remaining four for the tetracyclic system in **1**.

According to the ^{1}H–^{1}H COSY and HSQC spectra, one structural fragment of **1**, C-1/C-2/C-3(C-16)/C-4/C-5, was established, as shown in bold (Fig. 2). The locations of three methyls, Me-17, Me-19, and Me-20, were assigned to C-6, C-13, and C-10, respectively, based on HMBC correlations. Moreover, the following HMBC cross-peaks were diagnostic to assembling the A/B and B/C rings: 15-OH/C-4, C-15; H-1, H-14/C-15; H_3-20/C-6, C-12, C-13; H_3-17/C-5, C-6, C-7, C-13. The following HMBC cross-peaks obtained the C/D ring substructures H-11, H-18/C-8, and H-11/C-12 (Fig. 2). The 1,2,3,5-four-substituted aromatic ring D was established by the HMBC correlations of H_3-19 with C-11 and C-18. For the acylation pattern of Compound **1**, HMBC correlations of the three quaternary carbons (δ_C 166.5, 171.4, 170.0) with protons (H-3, H-5, H-14) helped establish *O*-functionalities at C-3, C-5, and C-14, respectively. Finally, the remaining two hydroxy groups were attached to quaternary carbons C-9 and C-15 due to their chemical shifts at 153.0 and 83.5, respectively. Thus, we determined the planar structure of **1** to be a pepluane-type diterpenoid with a rare aromatic ring D, as shown in Fig. 1. To our knowledge, there are only two pepluane-type diterpenoid compounds with a rare aromatic ring D reported to date [18].

The relative configuration of Compound **1** was elucidated from the ROE interactions observed in a ROESY experiment (Fig. 2). The ROESY correlations of H-4α/H-17/H-20 and H-3/H-4α established that H-3, H-17, and H-20 were α-oriented. The ROESY correlations of H-5β/H-14/OH-15/Me-16 revealed that the relative configurations of H-14, OH-15, and Me-16 were β-oriented. The structure of **1** was finally confirmed by single-crystal X-ray diffraction, which also unambiguously determined the absolute configuration to be 2*R*,3*S*,4*S*,5*R*,6*R*,13*S*,14*R*,15*R* with a Flack parameter of 0.12 (8) (Fig. 3).

Structure comparisons of **1**–**5** suggested a possible route for the biosynthesis of **1**. Herein, a plausible biosynthetic pathway for compound **1** is proposed (Scheme 1). Compounds **1**–**5** may be derived from the precursor of 5,8,14-triacetoxy-3-benzoyloxy-15-hydroxy-9-oxoparaliane (**6**) [26], which was also isolated from this plant, the reduction of which could lead to the generation of intermediate **7**. The presence of one less methyl in pepluane compared to paraliane suggested that Me-19 in the latter was incorporated in the ring system of the former. The oxidation of a methyl group at C-18 would give intermediate **7** for rearrangement, leading to appropriate pepluane **8**, which could be converted to compounds **3**–**5** via different esterifications. Then, intermediate **8** could form an α,β-unsaturated ketone moiety in **2** via dehydration and oxidization, followed by aromatization to produce **1**. To verify the rationality of the aromatization step in their biogenetic pathway, we treated Compound **2** with p-toluenesulfonic acid under reflux, which could produce **1** with a yield of 93% (Fig. 4).

By comparison of spectroscopic data with the literature, the four known compounds were determined to be pepluanol G (**2**) [6], 5,8,11,15-tetraacetoxy-3-benzoyloxy-9,16-dihydroxypepluane (**3**) [6], 5,8,9,11,15-pentaacetoxy-3-benzoyloxy-16-hydroxypepluane (**4**) and 5,8,9,15-tetraacetoxy-3-benzoyloxy-11,16-dihydroxypepluane (**5**) [1].

Compounds **1–5** were first evaluated for cytotoxicity. As shown in Fig. 5, none of the compounds exhibited toxicity between 0 and 40 μM. Then, these compounds were investigated for their anti-inflammatory activities against NO release from mouse RAW264.7 macrophages induced by LPS with dexamethasone (DXM) as a positive control. The results show that Compound **1** has a strong inhibitory effect on the release of NO from mouse macrophages caused by LPS (Fig. 6) in a dose-dependent manner, which at 20 μM was better than the positive control. Compounds **1** and **3** inhibited NO production in a dose-dependent manner (Fig. 7).

In this study, one new pepluane-type diterpenoid, pepluenone (**1**), and four known ones were isolated from the whole plant of *E. peplus* L. Compound **1** possesses a rare aromatic ring D, and its

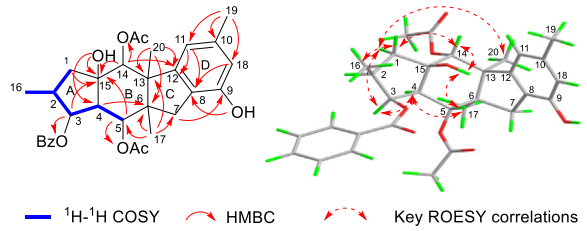


Fig. 2. Key HMBC, ^{1}H–^{1}H COSY, and ROESY correlation structures of 1.

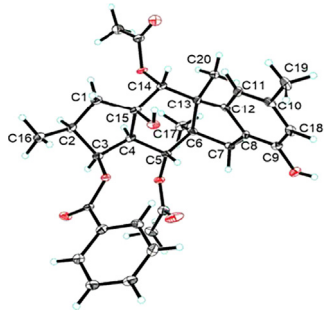
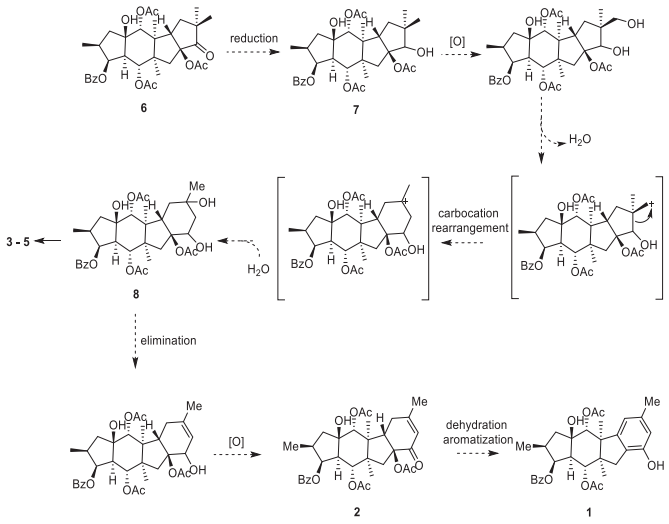


Fig. 3. ORTEP revealing the structure of 1.



Scheme 1. Proposed biogenetic relationship of Compounds **1–5**.

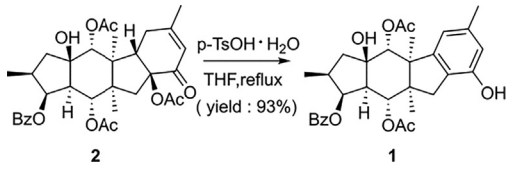


Fig. 4. Chemical transformation of Compound 2 to 1.

structure and absolute configuration were confirmed by single-crystal X-ray diffraction. The biogenetic pathway of **1** was proposed; in particular, we verified the construction of its aromatic ring D via chemical conversion. Moreover, **1** and **3** significantly inhibit NO release from mouse macrophages caused by LPS.

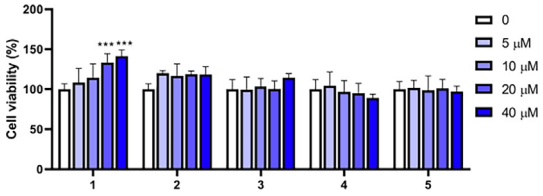


Fig. 5. The cytotoxicity of Compounds 1–5 on RAW264.7 macrophages. Data are expressed as the mean ± SD, ***p < 0.001, 0 vs. the indicated concentrations.

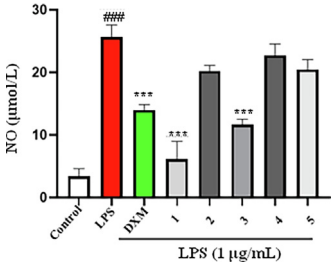


Fig. 6. Effect of Compounds 1–5 at 20 μM on NO production from mouse macrophage RAW264.7 cells induced by LPS. Data are expressed as the mean ± SD, ###p < 0.001, the control group vs. the LPS group; ***p < 0.001, the compound treatment group vs. the LPS group.

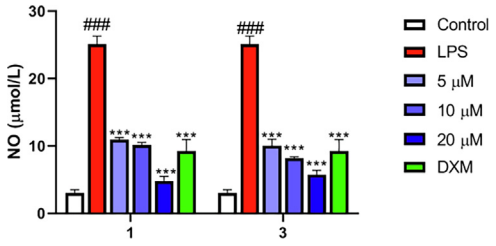


Fig. 7. Effect of Compounds 1 and 3 at 5, 10 and 20 μM on NO production from mouse macrophage RAW264.7 cells induced by LPS. Data are expressed as the mean ± SD, ###p < 0.001, the control group vs. the LPS group; ***p < 0.001, the compound treatment group vs. the LPS group.

Data availability

The data that has been used is confidential.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2023.154550.

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