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SHORT REPORT

Uvaol-28-D-(*R*)-pyroglutamic acid ester: a new triterpenoid compound from *Circinella muscae CGMCC 3.2695* and its activity assessment in transgenic zebrafish

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Abstract: A novel triterpenoid compound named uvaol-28-D-(*R*)-protoglutamic acid ester (UPE) was obtained via microbial biotransformation of uvaol by *Circinella muscae CGMCC 3.2695*. Its structure was elucidated by spectroscopic and mass-spectrometric analyses, including 1D- and 2D-NMR, HR-ESI-MS, and quant. UPE administration promotes intersegmental vessel (ISV) growth and motor neuron development in transgenic zebrafish embryos in a concentration-dependent manner. Furthermore, UPE significantly restored the swimming distance in zebrafish exposed to combretastatin A-4 (CA-4), as assessed by ESOvision behavioral analysis. These findings suggest that UPE holds promise for the treatment of cardiovascular and cerebrovascular diseases.

Keywords: Uvaol-28-D-(*R*)-pyroglutamic acid ester, *Circinella muscae CGMCC 3.2695*, transgenic zebrafish, intersegmental vessel, motor neuronal axonal

Cite this article as:
Zhang et al. Uvaol-28-D-(*R*)-pyroglutamic acid ester: a new triterpenoid compound from *Circinella muscae CGMCC 3.2695* and its activity assessment in transgenic zebrafish. (2026). *Records of Natural Products*, 20(1):e25083620

Received: *23 August 2025*
Revised: *17 November 2025*
Accepted: *17 November 2025*
Published: *03 December 2025*

1 Fungal Source

The fungi *Circinella muscae CGMCC 3.2695* were purchased from China General Microbiological Culture Collection Center (CGMCC), and were maintained at 4 °C on potato slants solidified with agar.

2 Previous Studies

Microbial biotransformation has emerged as a powerful strategy for generating structurally diverse derivatives of natural products. This process utilizes microbial enzymes to modify exogenous substrates, yielding novel metabolites with enhanced or altered bioactivities. Under the catalytic conditions provided by *Circinella muscae CGMCC 3.2695*, a range of biotransformed products has been successfully obtained (Wang et al., 2025; Chu et al., 2021; Lu et al., 2022). The triterpenoids are the largest and most structurally diverse terpenoids. Uvaol, a pentacyclic triterpene present in olives and olive oil, has multiple beneficial functions, including antioxidant, anti-inflammatory, and ameliorates lipid deposition, accelerates the healing of cutaneous wounds (Sánchez-Quesada et al., 2013; Pan et al., 2024; Gwon et al., 2024; Carmo et al., 2020). Some structural modifications using uvaol as a substrate have been completed, and new or activity-enhanced derivatives have been obtained (Cristoni et al., 1994).

3 Present Study

In this study, we employed *Circinella muscae CGMCC 3.2695* to biotransform uvaol, resulting in the isolation of a new triterpenoid derivative, uvaol-28-D-(*R*)-pyroglutamic acid ester (UPE) (Figure 1), as well as previously identified three compounds (compounds **2–4**) (Wang et al., 2024) (NMR data for compounds **2–4** see Table S1).

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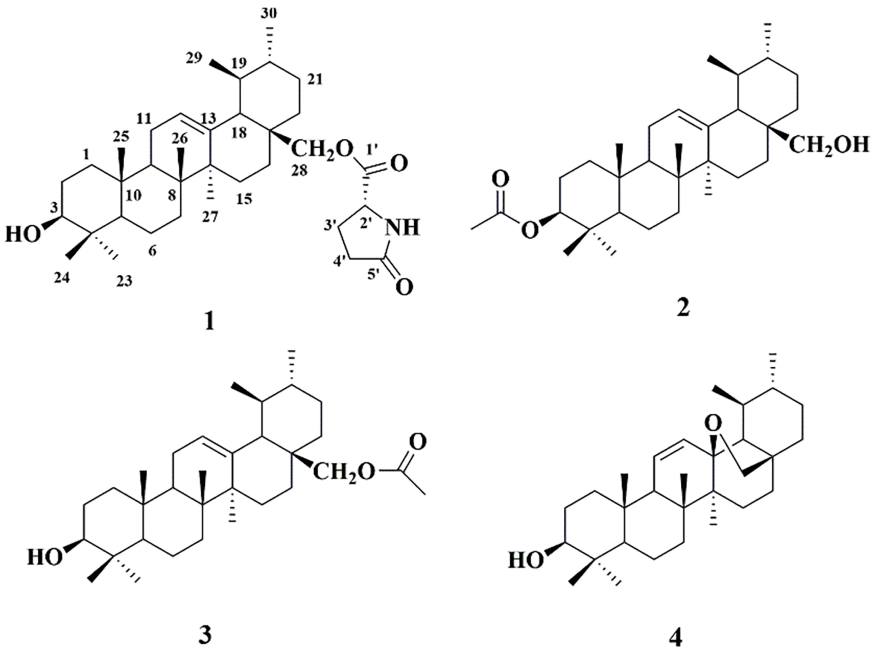


Figure 1. The structure of compounds **1** (UPE) and compounds **2–4**

Compound **1** (UPE) displayed a $[M+Na]^+$ HR-ESI-MS ion at *m/z* 576.4048 (calcd. $C_{35}H_{55}NO_4Na$, 576.4029, Figure S7), which is 111 amu heavier than uvaol, indicating a molecular formula of $C_{35}H_{55}NO_4$. The ^{1}H-NMR spectrum (Table 1, Figure S1) of UPE displays two doublets at δ_H 3.70 and 4.19 for the C28A and C28B protons, which are ca. 0.5 and 0.7 ppm more downfield shifted than those of the C28A and B protons of uvaol (i.e., Figure S14; δ_H 3.20 and 3.53, respectively). These differences suggest that the hydroxyl group of UPE located at the C28 methyl group should be esterified, comparison of the ^{1}H-NMR data with those of uvaol-28-acetyl ester (**3**) (Table S1) and uvaol-28-formyl ester (UFE) (Table S2) (Lee et al., 2013) with almost identical C28A and C28B proton chemical shifts strongly corroborates the presence of an ester group at the C28 hydroxyl group in UPE (**1**). Furthermore, HMBC correlations observed between the H-2′ proton (at δ_H 4.25) and C-1′ (δ_C 171.9), and C-5′ (δ_C 177.7); as well as between the H-28A and B protons (at δ_H 3.70 and 4.19) and

Table 1. NMR data for compound UPE in $CDCl_3$ (δ in ppm and *J* in Hz)

No.	Uvaol-28-D-(*R*)-pyroglutamic acid ester	
	δ_H (*J* in Hz)	δ_C
1	1.01 m, 1.64 m	38.9
2	1.60 m	27.2
3	3.21 dd (11.3, 4.8)	79.2
4	–	38.9
5	0.72 bd (11.6)	55.6
6	1.38 m, 1.52 m	18.3
7	1.32 m, 1.52 m	32.7
8	–	40.1
9	1.50 m	47.6
10	–	36.9
11	1.91 m	23.6
12	5.15 t (3.4)	126.1
13	–	138.0
14	–	42.1
15	1.01 m, 1.70 m	26.1
16	1.17 bd (13.6), 1.98 td (4.8, 13.6)	23.6
17	–	37.0
18	1.41 m	54.3

http://doi.org/10.25135/rnp.2508.3620
Rec. Nat. Prod. 2026, 20(1):e25083620

Table 1. (Continued)

No.	Uvaol-28-D-(*R*)-pyroglutamic acid ester	
	δ_H (*J* in Hz)	δ_C
19	1.39 m	39.4
20	0.88 m	39.5
21	1.32 m, 1.52 m	32.9
22	1.32 m, 1.54 m	35.9
23	1.00 s	28.1
24	0.79 s	15.7
25	0.95 s	15.6
26	0.98 s	16.7
27	1.10 s	23.5
28	3.70 d (10.9), 4.19 d (10.9)	72.5
29	0.81 d (4.6)	17.4
30	0.93 d (5.2)	21.4
1′	–	172.0
2′	4.25 dd (8.7, 5.3)	55.6
3′	2.21 m, 2.50 m	25.2
4′	2.33 m, 2.37 m	29.4
5′	–	177.7

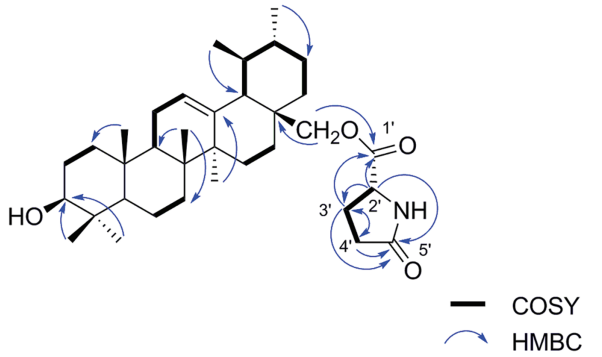
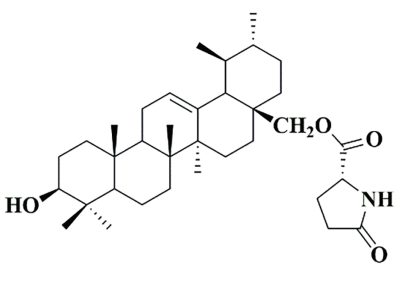
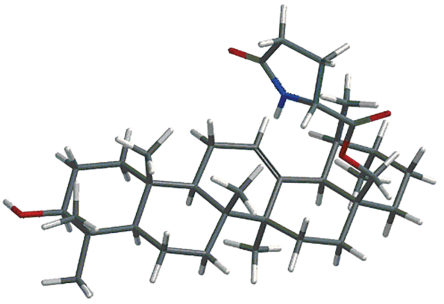


Figure 2. Selected ^{1}H-^{1}H COSY and HMBC correlations of compound UPE (**1**)

C-1′ (δ_C 171.9) carbonyl signal in the HMBC spectrum of UPE (Figures 2 and S6) confirms the presence of an ester group at the C28 hydroxymethyl group. In addition to the aforementioned HMBC correlations, close examination of the HMBC spectrum of UPE (Figures 2 and S6) shows a correlation between H-2′ (at δ_H 4.25) and C-3′ (δ_C 25.2), between H-4′ protons (δ_H 2.33 and 2.37) and C-5′ (δ_C 177.7), and reciprocal correlations between H-3′ protons (δ_H 2.21 and 2.50) and C-4′ (δ_C 29.4) as well as H-4′ protons (δ_H 2.33 and 2.37) and C-3′ (δ_C 25.2) indicating the presence of a protoglutamyl group as the ester group attached at the C-28 hydroxymethyl group of UPE (**1**). In addition, the proton-proton correlations between H-2′, H-3′, and H-4′ protons in the COSY spectrum of UPE (**1**) (Figures 2 and S4) confirm the presence of a pyroglutamic acid ester group in UPE.

Table 2. Summary of the DP4+ quantum-chemistry calculation results of UPE-2′-isomers





	DP4+ probability (%)	RMS	Mean
***R*-isomer**	**97.3**	4.44	2.78
S-isomer	2.7	4.57	2.95

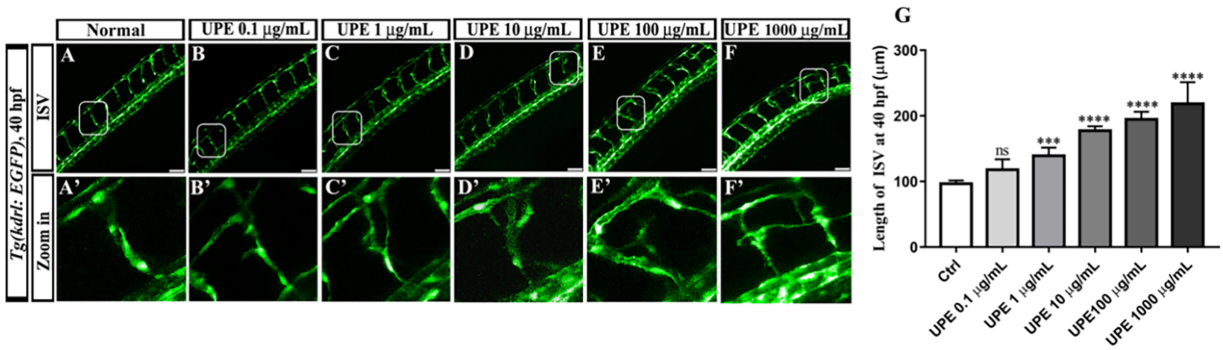


Figure 3. Effects of UPE on *Tg (kdrl:EGFP)* embryonic zebrafish. (A–F) ISV phenotypes of control group, and different concentrations of UPE (0.1, 1, 10, 100, and 1000 μg/mL) treated groups at 40 hpf. (A′–F′) Zoom in of A–F. (G) Statistical analyses of ISV length (n ≥ 6). ***$p < 0.001$ vs normal group, ****$p < 0.0001$, vs normal group. Scale bar 75 μM. The data are presented as the mean ± SD

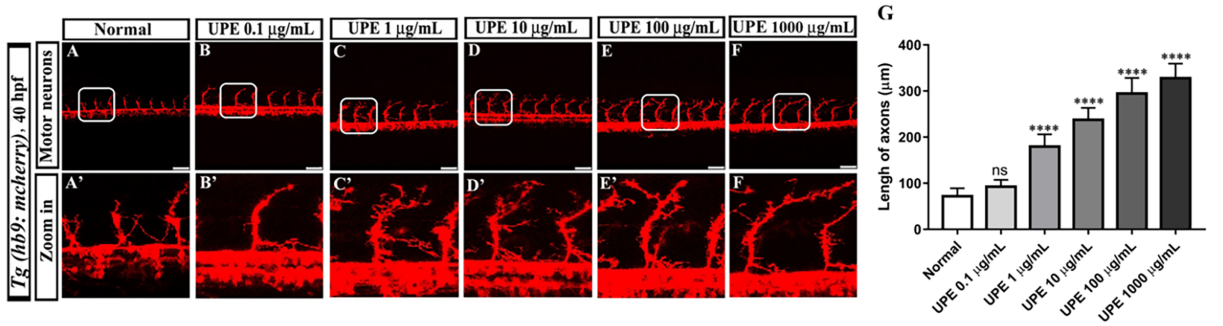


Figure 4. Effects of UPE on *Tg (hb9:mcherry)* embryonic zebrafish motor neuronal axons. (A–F) Phenotype of motor neuron by UPE (0.1, 1, 10, 100, and 1000 μg/mL) treatment at 40 hpf. (A′–F′) Zoom in of A–F. (G) Statistics of the neuronal axons length (n ≥ 6). ****$p < 0.0001$, vs normal group. Scale bar 75 μM. The data are presented as the mean ± SD

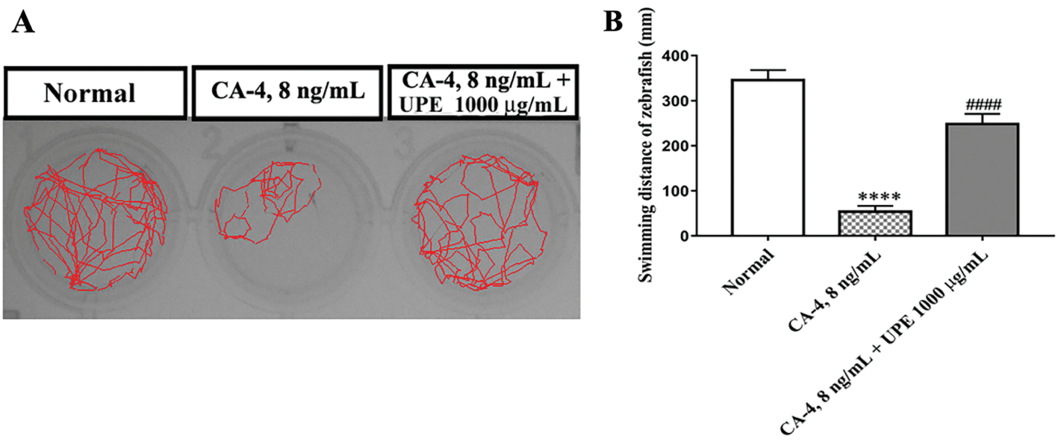


Figure 5. ESOvision behavioral analysis for swimming distance of zebrafish at 120 hpf (swimming behavior assay (3 min)). (A) ESOvision behavioral analysis software for swimming distance by normal, CA-4 (8 ng/mL), CA-4 (8 ng/mL) + UPE (1000 μg/mL) treatments. (B) Statistics of the swimming distance (n ≥ 6). ****$p < 0.0001$, vs normal group. $^{\#\#\#\#}p < 0.0001$, vs CA-4 (8 ng/mL) group. The data are presented as the mean ± SD

To unequivocally identify the chirality of the C-2′ asymmetric center of the pyroglutamic acid ester group, we performed quantum-chemical calculations to determine the exact stereochemistry of UPE (**1**). Following the determination of the lowest-energy conformers of UPE-2′-(*R*)- and UPE-2′-(*S*)-pyroglutamate isomers, ^{13}C-NMR theoretical chemical shifts for both (*R*)- and (*S*)-isomers were calculated (Figure S17). Then, the DP4+ analysis was performed by comparing the theoretically calculated ^{13}C-NMR chemical shifts with the experimental ^{13}C-NMR chemical shift results (Table S3) (Hehre et al., 2019). The DP4+

http://doi.org/10.25135/rnp.2508.3620
Rec. Nat. Prod. 2026, 20(1):e25083620

statistical analysis results strongly suggest that the structure of UPE (**1**) is uvaol-28-D-(*R*)-pyroglutamic acid ester (Table 2).

The zebrafish model was used to assess the activity of UPE. The effect of UPE on angiogenesis and motor neurons was evaluated using *Tg (kdrl: EGFP)* and *Tg (hb9: EGFP)* zebrafish, respectively. In addition, the swimming distance experiment of zebrafish was further used to observe the effect of UPE on zebrafish behavior. Combretastatin A-4 (CA-4), a chemotherapy drug, can induce neuronal damage in zebrafish, thereby reducing their swimming distance (Shi et al., 2016).

The results demonstrated UPE treatment with 1, 10, 100, and 1000 μg/mL significantly increased the length of ISV of zebrafish at 40 hpf ($p = 0.0006$, $p < 0.0001$, $p < 0.0001$, $p < 0.0001$, respectively) (Figure 3). Similarly, UPE treatment with 1, 10, 100 μg/mL, and 1 mg/mL significantly promoted neuronal development in a concentration-dependent manner at 40 hpf ($p < 0.0001$, $p < 0.0001$, $p < 0.0001$, $p < 0.0001$, respectively) (Figure 4). The behavioral results displayed that CA-4 (8 ng/mL) administration significantly shortened the swimming distance of zebrafish compared with the normal group, and compared with the CA-4 (8 ng/mL) treatment group, the swimming distance of zebrafish in the CA-4 (8 ng/mL)+UPE (1000 μg/mL) treatment group significantly increased (Figure 5). The effect of UPE on promoting nerve growth may be related to its promotion of angiogenesis as the blood vessels provide nutrients, immunity and other substances for nerve growth and development (Wang et al., 2020).

In summary, our findings demonstrate that UPE promotes angiogenesis and motor neuron development and ameliorates behavioral impairments in zebrafish. These results highlight the therapeutic potential of UPE in the treatment of cardiovascular and neurodegenerative diseases.

Funding Statement

This work was partially supported by Nantong Municipal Health Commission (MSZ2023026 to X. Z.), Nantong Social Livelihood Science and Technology Project (MS2024037 to W. C.), Key Project of Special Scientific Research Fund for Clinical Medicine of Nantong University (2023JZ001 to W. C.), and Nantong Project of Science and Technology (MS2024066 to X. T.).

Author Contributions

Conceptualization: Weiguan Chen and Xiaofang Tan. Methodology: Xiaoli Zhang, Xiaoling Jin, Hao Li and Mahmut Miski. Investigation: Weiguan Chen and Xiaofang Tan. Formal analysis: Weiguan Chen, Xiaofang Tan and Mahmut Miski. Resources: Weiguan Chen and Xiaofang Tan. Writing—Original Draft: Weiguan Chen and Xiaofang Tan. Project administration: Weiguan Chen and Xiaofang Tan.

Availability of Data and Materials

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Ethics Approval

This work is approved by the Animal Protection Ethics Committee of Nantong University, China (Approval Number: S20240715-006).

Conflicts of Interest

The authors declare that they do not have any conflict of interest.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products.

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