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Synthesis of new derivatives of alepterolic acid *via* click chemistry

XIN JIN[1,2], JIANGUO CAO[3], QINGJIE ZHAO[4], QI WANG[3], HONGMEI GUO[1,2], HAJI AKBER AISA[1]* and GUOZHENG HUANG[1,5]**

[1]*Key Laboratory of Plant Resources and Chemistry of Arid Zone, Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Urumqi, 830011, P.R. China,* [2]*University of Chinese Academy of Sciences, Beijing, 100049, P.R. China,* [3]*College of Life Sciences, Shanghai Normal University, Shanghai, 201418, P.R. China,* [4]*CAS Key Laboratory for Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, P. R. China and* [5]*College of Chemistry and Chemical Engineering, Anhui University of Technology, Ma'anshan, 243002, P. R. China*

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Abstract: Alepterolic acid is a natural diterpenoid isolated from *Aleuritopteris argentea* (S. G. Gmél.) Fée, a fern with potential medicinal activity, used in China as a folk medicine to regulate menstruation and prevent cancer. Nevertheless, there are few reports about the structural modification of this natural product. With the wide application of 1,2,3-triazole derivatives in medicines, pesticides, functional materials, the synthesis of 1,2,3-triazoles derivatives has attracted the attention of synthetic chemists. In this article, 23 new derivatives of alepterolic acid combined with 1,2,3-triazole were designed and synthesized by esterification and click chemistry reaction in a fast, conventional and efficient way. All the products were obtained in good yields (72 to 97 %). The structure of these compounds was confirmed by ^{1}H-, ^{13}C-NMR and mass spectral data. The use of the easily available reactants and the common reaction conditions furnish an efficient method for the synthesis of alepterolic acid derivatives. The preparation of these compounds would enable further biological evaluation in the future.

Keywords: diterpenoid; 1,2,3-triazole; structural modification.

INTRODUCTION

Aleuritopteris argentea (S. G. Gmél.) Fée, also known as *Cheilanthes argentea* (S. G. Gmél.) Kunze, is a kind of medicinal fern plant growing in China, Japan, the Korean Peninsula and Russia. Extracts from the fern possess a wide range of medicinal values, such as promoting blood circulation, regulating mens-

*,** Corresponding authors. E-mail: (*)haji@ms.xjb.ac.cn; (**)guozheng.huang@ahut.edu.cn
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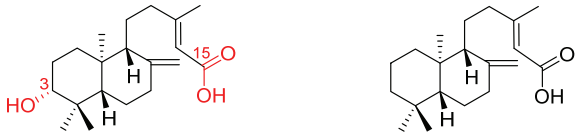
truation, tonifying asthenia and relieving cough. In 1962, Ageta *et al.* isolated a diterpenoid compound from *A. argentea* for the first time, namely alepterolic acid (Fig. 1).[1] Later, it was found that alepterolic acid was produced in specimens of *A. argentea* from Japan and mainland Asia, while *ent*-anti-copalic acid was present (Fig. 1) in specimens from Taiwan island. Thus, the types and content of metabolites of this fern are different according to the geographical distribution.[2] These two diterpenoids share the same labdane frame, with a hydroxy group at 3-positon in the molecule of alepterolic acid. Recently, Idippily *et al.* reported the structural modification of *ent*-anti-copalic acid by amination and esterification reactions. Compared to that of *ent*-anti-copalic, the anticancer activity of those semi-synthesized derivatives against LNCaP cell line considerably increased.[3] However, the modification of alepterolic acid was less investigated.



Alepterolic acid **ent-anti-Copalic acid**

Fig. 1. Structure of alepterolic acid and *ent*-anti-copalic acid.

1,2,3-Triazole, as one of the most important heterocycles, can form various non-covalent interactions such as hydrophobic interactions, hydrogen bonds, van der Waals forces with different biological targets.[4] Accordingly, the compounds with 1,2,3-triazole moiety possess diverse pharmaceutical properties such as antibacterial,[5] antimalarial,[6] antifungal,[7] antiviral,[8] antitubercular[9] and anticancer[10] activities. The combination of 1,2,3-triazole with other bioactive drug molecules or natural products may produce hybrid molecules with better biological activity performance.[11,12] Previously, our group reported the structural modification of rupestonic acid (Fig. 2) by the introduction of the 1,2,3-triazole. The synthesized derivatives displayed interesting potency against influenza A viruses.[13] Carnosic acid (Fig. 2), a diterpenoid, exhibited potential antiproliferative and antifungal activities after combination with 1,2,3-triazole by Pertino *et al.*[14] Acanthoic acid (Fig. 2), also a kind of diterpenoid compound, was recently modified by Kasemsuk, with the results demonstrating that the triazole ring and nitro group on the benzyl ring play a pivotal role in the cytotoxic activity against cholangiocarcinoma.[15] It was reported that hybrids of oleanolic acid (Fig. 2) and 1,2,3-triazole possess considerable anticancer activity and could inhibit the proliferation of a variety of tumor cells.[16] Based on the above-mentioned studies, the introduction of 1,2,3-triazole moiety into alepterolic acid is supposed to be a prospective derivatization method to acquire or enhance the bioactivities.

Our group recently isolated grams of alepterolic acid from *A. argentea*. A pioneer derivation was achieved by incorporation of amino moiety to the 15-carboxy group.[17] The obtained compounds showed improved cytotoxic activity against HeLa cell lines compared to alepterolic acid itself. Taking these factors into account, we intended to further modify alepterolic acid by introducing 1,2,3--triazole moiety.

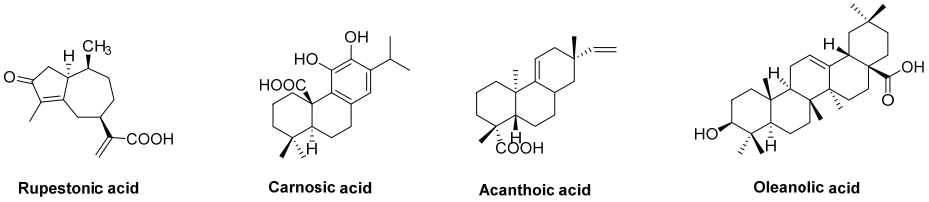


Fig. 2. Structures of rupestonic acid, carnosic acid, acanthoic acid and oleanolic acid.

EXPERIMENTAL

Visualization on TLC was achieved by use of UV light (254 nm) or iodine. NMR spectra were recorded on a Varian Inova-400 MHz spectrometer (400 MHz for ^{1}H, 100 MHz ^{13}C). The spectra were recorded in $CDCl_3$ as solvent at room temperature, ^{1}H- and ^{13}C-NMR chemical shifts are reported in ppm relative to either the residual solvent peak (^{13}C, δ = 77.16 ppm or ^{1}H, δ = 7.26 ppm) as an internal standard. Data for ^{1}H-NMR are reported as follows: chemical shift (δ / ppm), multiplicity, integration, coupling constant (Hz) and assignment. The melting point of the product was determined by Buchi M-560 melting point apparatus.

Propargyl bromide, NaN_3, $CuSO_4 \cdot 5H_2O$, substitued benzyl bromides and anilines are purchased from Adamas Reagent, Co. Ltd. (Shanghai, China) and were used without further purification.

*Synthesis of prop-2-yn-1-yl (*E*)-5-((1*R*,4a*S*,6*R*,8a*S*)-6-hydroxy-5,5,8a-trimethyl-2-methylene-decahydronaphthalen-1-yl)-3-methylpent-2-enoate (**3**)*

To the solution of alepterolic acid (960.7 mg, 3.0 mmol) in 10 mL of DMF was added potassium carbonate (621.9 mg, 4.5 mmol) and propargyl bromide (428.3 mg, 3.6 mmol). The reaction was stirred at room temperature for 12 hours. Then the mixture was quenched with water and extracted with ethyl acetate (3×10 mL). Combined organic layers were washed with water (3×20 mL), brine (20 mL), and dried over Na_2SO_4. After the solvent was removed in vacuum, the crude product was purified by column chromatography (petroleum ether:ethyl acetate, 4:1 to 1:1 volume ratios) to afford 969.1 mg of desired product as a yellow solid (yield 90 %).

*General procedure for the synthesis of substituted phenyl azides **4a**–**n***

To a stirred solution of substituted aniline (1.0 mmol) in 2 mL of 6 N HCl at 0 °C was added $NaNO_2$ (103.5 mg, 1.5 mmol) in H_2O (2 mL). After stirring for 15 min, a solution of NaN_3 (78 mg, 1.2 mmol) in H_2O (1 mL) was cautiously added dropwise. The reaction was left to stir for 1 h at room temperature, followed by extraction with ethyl acetate (3×5 mL). The combined organic layers were dried over Na_2SO_4, and carefully concentrated under reduced pressure to give corresponding phenyl azide. The crude product was used directly without purification.

*General procedure for the synthesis of substituted benzyl azides **4o**–**w***

To a stirred solution of substituted benzyl bromide (1.0 mmol) in 4 mL of acetone was added NaN_3 (96 mg, 1.50 mmol) in 1 mL of water. After stirring for 12 h, the reaction mixture was extracted with ethyl acetate (3×10 mL), washed with brine, dried over Na_2SO_4, and concentrated carefully under reduced pressure to give corresponding benzyl azide. The crude product was used directly without purification.

*General procedure for the synthesis of 1,2,3-triazoles derivations of alepterolic acid **5a**–**w***

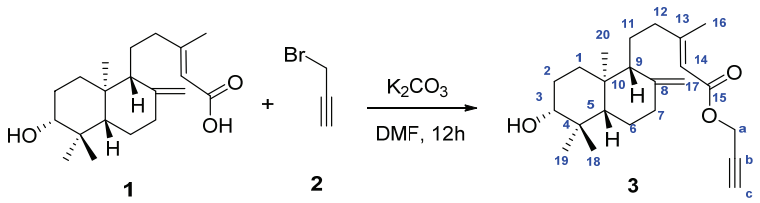
To the solution of compound **3** (30.0 mg, 0.083 mmol) and corresponding azide (0.10 mmol) in 4 mL of DMF/H_2O (1:1 volume ratio) was added sodium ascorbate (6.5 mg, 0.033 mmol) and $CuSO_4 \cdot 5H_2O$ (3.8 mg, 0.014 mmol). The reaction was stirred at 60 °C for 6 h and monitored by thin layer chromatography to ensure that compound **3** had been completely consumed. Then the mixture was quenched with ice-cold water and extracted with ethyl acetate (3×5 mL). Combined organic layers were washed with water (3×5 mL), brine (10 mL), and dried over Na_2SO_4. After the solvent was removed in vacuum, the crude product was purified by column chromatography (petroleum ether:ethyl acetate, 1:1 to 1:4 volume ratios) to afford the desired products **5a**–**w**.

The detailed spectral data of synthetic compounds are presented in the Supplemental material to this paper.

RESULTS AND DISCUSSION

Click chemistry is applied to all aspects of drug discovery to form 1,2,3-triazole compounds. The copper-(I)-catalyzed 1,2,3-triazole formation from azides and terminal acetylenes is a very useful route to create a library of new compounds for the screening of activity, due to its high degree of dependability, complete specificity and the biocompatibility of the reactants.[18] With that in mind, we used classical click reaction conditions to prepare a series of 1,2,3-triazole derivatives of alepterolic acid, taking copper sulfate pentahydrate and sodium ascorbate as the source of catalytic copper (I).

At the beginning, the substrate of the click reaction, propargyl alepterolate (**3**) was prepared by simple derivatization on alepterolic acid in yield of 90 % by mixing alepterolic acid (**1**) and 3-bromoprop-1-yne (**2**) with potassium carbonate in *N*,*N*-dimethylformamide for 12 h (Scheme 1).[19]



Scheme 1. Synthesis of propargyl alepterolate. Reaction condition: propargyl bromide, alepterolic acid, K_2CO_3, *N*,*N*-dimethylformamide, r.t.

Other components of the click reaction substrate, are phenyl azides and benzyl azides. Phenyl azides **4a**–**n** were synthesized by firstly mixing the corres-

ponding aniline, sodium nitrite and concentrated hydrochloride at 0 °C for 15 min, then adding sodium azide to the system and stirring at room temperature for 1 h.[20] The resulting products are listed in Table I. Benzyl azides **4o**–**w** were synthesized by mixing the corresponding benzyl bromide and sodium azide in a mixed solvent of acetone and water and reacting overnight.[21] The resulting products are listed in Table II. All the azides were obtained in high yields after simple work-up and directly used in the next step without further purification.

TABLE I. Preparation of phenyl azides **4a**–**n**; [a]reaction conditions: 1) $NaNO_2$, aniline, 6 M HCl, H_2O, 0 °C; 2) NaN_3, r.t., H_2O

$$\mathbf{R}NH_2 \xrightarrow[(2)\ NaN_3]{(1)\ HCl\ NaNO_2} \mathbf{R}N_3$$

Entry	Azide	R	Yield[a], %
1	**4a**	Ph	91
2	**4b**	2-MePh	75
3	**4c**	3-MePh	85
4	**4d**	4-MePh	90
5	**4e**	4-FPh	67
6	**4f**	4-ClPh	92
7	**4g**	4-BrPh	85
8	**4h**	4-MeOPh	77
9	**4i**	4-CNPh	93
10	**4j**	4-CF_3Ph	90
11	**4k**	4-NO_2Ph	62
12	**4l**	2,4-2ClPh	93
13	**4m**	3,4-2ClPh	80
14	**4n**	1-Nap	81

[a]Isolated yield

TABLE II. Preparation of benzyl azides 4o–4w; reaction conditions: NaN_3, benzyl bromide, acetone/ H_2O, r.t.

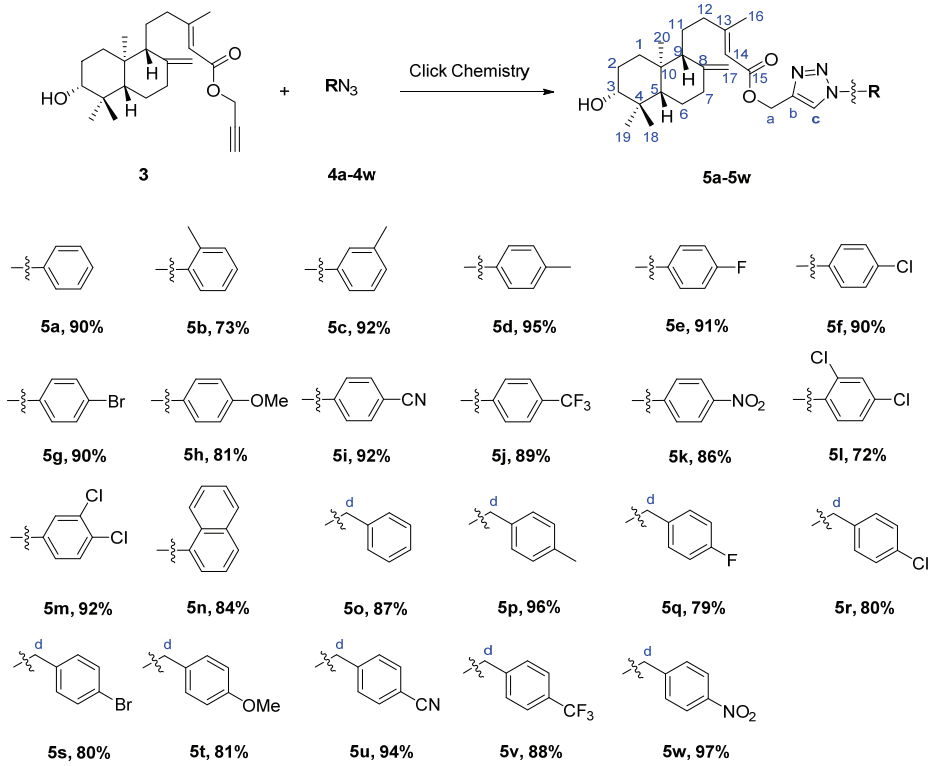
$$\mathbf{R}Br \xrightarrow[acetone\ /\ H_2O]{NaN_3} \mathbf{R}N_3$$

Entry	Azides	R	Yield[a], %
1	**4o**	Bn	95
2	**4p**	4-MeBn	94
3	**4q**	4-FBn	85
4	**4r**	4-ClBn	90
5	**4s**	4-BrBn	88
6	**4t**	4-MeOBn	88
7	**4u**	4-CNBn	85
8	**4v**	4-CF_3Bn	92
9	**4w**	4-NO_2Ph	95

[a]Isolated yield

The preparation of 1,2,3-triazoles derivatives of alepterolic acid **5a**–**w** were achieved as depicted in Scheme 2. All the products were prepared in good yields

as displayed. It is worth mentioning that when *ortho* substituted phenyl azides participated in the reactions such as **4b** or **4l**, the yields were relatively lower than with the other substrates, which implied that steric hindrance affected and limited the scope of the reaction to some extent similar to that previously reported.[22] Due to the existence of the ester group, most of the products stayed oily at room temperature.



Scheme 2. Synthesis of 1,2,3-triazoles derivtives of alepterolic acid. Reaction conditions: propargyl alepterolate, azides, sodium ascorbate, $CuSO_4 \cdot 5H_2O$, *N*,*N*-dimethylformamide/H_2O, 60 °C; isolated yield.

The structures of obtained compounds were analyzed by NMR and HRMS. The assignments of ^1H and ^{13}C spectra to the peak positions of the synthesized derivatives **5a**–**w** were attained by comparison with the signals to the original NMR data of alepterolic acid.[2,17] Compared with the ^1H-NMR of propargyl alepterolate, the terminal alkyne hydrogen signal disappeared, and the aromatic hydrogen atom signals appeared, while no obvious change was observed in the signal of methylene at the α-position of the ester group. The ^1H-NMR revealed the signal of hydrogen atom fluctuating from $\delta \approx 8.19$ to 7.51 ppm in the newly formed five-membered 1,2,3-triazole rings. The signal of 3-OH in the target com-

pounds appeared at $\delta \approx 3.24$ or 3.23 ppm, usually as a doublet of doublets, just like that of alepterolic acid, indicating that 3-OH was left untouched in the reactions. The signals of 14-H and 17-H on the NMR spectra were similar to those of alepterolic acid, which revealed the double-bond was tolerable for click chemistry. The signals of the four methyl groups stayed the same as those in alepterolic acid. From the information provided by ^{1}H-NMR, it can be concluded that the frame of alepterolic acid other than the carboxyl group was not affected during the whole process of the reactions. The ^{13}C-NMR data also consisted to the related structures, which tallied with previous literature reports.[2,17] The mass of the products showed molecular ion peaks corresponding to the molecular weight of the acquired structures.

CONCLUSIONS

Alepterolic acid, as the main metabolite of the fern plant *A. argentea*, is a diterpenoid worthy of structural modification. In this work, we have successfully developed a rapid and efficient process for the synthesis of 1,2,3-triazole derivatives through the application of click reaction. Twenty-three derivatives were synthesized in good yields and characterized by spectral analysis. These compounds would be evaluated with regard to their biological activities, such as anticancer or antiviral activities for future drug discovery.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/10525, or from the corresponding author on request.

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ИЗВОД

СИНТЕЗА НОВИХ ДЕРИВАТА АЛЕПТЕРОЛИЧНЕ КИСЕЛИНЕ ПРИМЕНОМ КЛИК-ХЕМИЈЕ

XIN JIN[1,2], JIANGUO CAO[3], QINGJIE ZHAO[4], QI WANG[2], HONGMEI GUO[1,2], HAJI AKBER AISA[1] и GUOZHENG HUANG[1,5]

[1]Key Laboratory of Plant Resources and Chemistry of Arid Zone, Xinjiang Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Urumqi, 830011, P.R. China; [2]University of Chinese Academy of Sciences, Beijing, 100049, P.R. China; [3]College of Life Sciences, Shanghai Normal University, Shanghai, 201418, P.R. China, [4]CAS Key Laboratory for Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, P. R. China, [5]College of Chemistry and Chemical Engineering, Anhui University of Technology, Ma'anshan, 243002, P. R. China

Алептеролична киселина је природни дитерпеноид изолован из папрати *Aleuritopteris argentea* (S.G. Gmél.) Fée, као једињење са потенцијалном медицинском применом, које се користи у кинеској народној медицини за регулацију менструалног циклуса и превенцију канцера. Ипак, постоји мало радова у којима су описане структурне моди-

фикације овог природног производа. Због широке примене 1,2,3-триазола у медицини, истраживању пестицида и материјала, постоји велико интересовање за синтезу деривата 1,2,3-триазола. У овом раду синтетисано је 23 деривата алептероличне киселине са 1,2,3-триазолским сегментом, применом реакција естерификације и "клик"-хемије, на брз, ефикасан и конвенционалан начин. Сви производи су добијени у добром приносу (од 72 до 97 %). Структуре једињења потврђене су ^{1}H-, ^{13}C-НМР и масеним спектрима. Употребом приступачних реагенаса под уобичајеним реакционим условима развијен је ефикасан поступак за синтезу деривата алептероличне киселине. Синтеза ових једињења ће омогућити даља биолошка испитивања у наредном периоду.

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