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Synthesis and Antimicrobial Activity of 8-Alkyl Coptisine Derivatives

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The compounds 8-butyl (**2**), 8-hexyl- (**3**), 8-octyl- (**4**), 8-decyl (**5**) and 8-dodecylcoptisine chloride (**6**) were synthesized and their antimicrobial activities were tested *in vitro*. LD_{50} were tested by mice's abdominal cavity injection in order to evaluate their structure-activity relationships. The results indicated that the derivatives exhibited high antimicrobial activities than coptisine, especially against Gram-positive bacteria and fungi. The 8-octylcoptisine displayed the highest antimicrobial activity in all compounds. The toxicity of compounds **2-6** was stronger than that of coptisine. However, upon elongating the aliphatic chain, the toxicity decreased gradually.

Key Words: Synthesis, 8-Alkyl-coptisine, Antimicrobial activity, Acute toxicity.

INTRODUCTION

Coptis chinensis is commonly known as a traditional Chinese medicinal plant. It is distributed in the south of China. Coptisine (**1**) is a kind of quaternary protoberberine alkaloid (QPA)[1] and one of the active constituents of *Coptis chinensis* Franch[2]. It has hypoglycemic and antidiabetic[3] and antioxidant[4], gastric-mucous membrane protection activity[5], antibacteria activity[6]. Substituted derivatives of quaternary protoberberine alkaloid in the A, C or D ring exhibit changes in their pharmacological effects. Iwasa[7] reported that dioxymethylene replacement at the C-2 and C-3 positions in the A ring, as well as 8-alkyl- or 13-alkyl-substitution[8,9] increased, but 13-hydroxy-substituted derivatives decreased their antibacterial activity[10]. Duk[11] reported that benzyl introduced to 13-C of berberine and berberrubine increased the antifungal activities. The antimicrobial activities of 8-alkylberberine derivatives increased with the aliphatic chain elongation and then decreased gradually when the alkyl chain exceeds eight carbon atoms[12]. In this study, we synthesized a series of 8-alkylcoptisine derivatives by introducing alkyl groups at C-8 of coptisine and their antimicrobial activities were evaluated *in vitro* and LD_{50} were evaluated by Mice's abdominal cavity injection to study their structure-activity relationships.

EXPERIMENTAL

Melting points were determined on an RD-2C electrothermal melting point apparatus and are uncorrected. The ^1H and ^{13}C NMR spectra were recorded on a Bruker Model Avance DMX300 using TMS as the internal standard and DMSO-d_6 as solvent. TLC analysis was used to confirm the purity of the compounds, which was performed on silica gel-GF254 thin layers and developed with a moving phase of C_6H_6/EtOAc/MeOH/iC_3H_7OH/NH_3 (6:3:1.5:1.5:0.5).

The coptisine used for synthesis was extracted from *Rhizoma coptidis*. It's purity was more than 98.5 % analyzed by HPLC.

The 8-alkycoptisine derivatives were synthesized according to the previous report[13]. Grignard reagents were prepared from Mg ribbon (8.8 mmol) and the corresponding alkyl bromides (8 mmol) in absolute THF (10 mL) were slowly added to the suspension of dry coptisine chloride (2 mmol) in absolute THF (10 mL) under nitrogen atmosphere at 0 °C. After 2 h of reflux, the 8-alkyl-coptisine bromides were obtained. A mixture of hydrobromides, Br_2 (1.8 mmol) and THF (30 mL) was heated at 50 °C and no further change in the composition of the reaction mixture was evident by TLC. After cooling, the precipitates were filtered and washed with 10 % $Na_2S_2O_5$ solution and H_2O, respectively. The crude the 8-alkyl-coptisine bromides were obtained, which were crystallized from MeOH. Thereafter, these bromides were added into hot MeOH containing AgCl (1.8 mmol) and converted into corresponding chlorides. Melting points, ^1H and ^{13}C NMR and TLC were used to identify the structures of compounds **1-6**.

Compound 1: It is yellow power , R_f 0.76 silica gel-GF_{254} C_6H_6/EtOAc/MeOH/iC_3H_7OH/NH_3 (6:3:1.5:1.5:0.5). ^1H NMR

(300 MHz, DSMO-d_6): δ 9.91 (1H, s, H-8), 8.92 (1H, s, H-13), 8.02 (1H, d, J = 8.0 Hz, H-11), 7.81 (1H, d, J = 8.0 Hz, H-12), 7.77 (1H, s, H-1), 7.07 (1H, s, H-4), 6.52 (2H, s, OCH_2O), 6.16 (2H, s, OCH_2O), 4.86 (2H, m, H-6), 3.18 (2H, m, H-5); ^{13}C NMR (100 MHz,CD_3OD): δ 147.1 (C-10), 149.8 (C-3), 147.7.1 (C-2), 144.6 (C-8), 143.9 (C-9), 136.9 (C-13a), 132.4 (C-12a), 130.6 (C-4a), 121.8 (C-12), 120.5 (C-13b), 121.1 (C-13), 121.0 (C-11), 111.7 (C-8a), 108.5 (C-4), 105.4 (C-1), 104.05 (OCH_2O), 102.1 (OCH_2O), 55.2 (C-6), 26.3 (C-5). Dept 135 show four methylenes signal: 104.05 (OCH_2O), 102.1 (OCH_2O) 55.2 (C-6), 26.3 (C-5) six methylene blue signal: 108.5 (C-4) 144.6 (C-8) 121.0 (C-11) 121.8 (C-12) 105.4 (C-1) 121.1 (C-13), ESIMS m/z: $[M+1]^+$ = 319.66, m.p. 218 ºC. the above data is basically the same to literature[13] reported, compound **1** is coptisine.

Compound 2: It was obtained as russet flakes from MeOH R_f 0.85 silica gel-GF_{254} C_6H_6/EtOAc/MeOH/iC_3H_7OH/NH_3 (6:3:1.5:1.5:0.5). ^{1}H NMR (300 MHz, DSMO-d_6): δ 1.16 (t, J = 6.8 Hz, 3H, -CH_3), 1.55 [m, 2H, -CH_2-], 1.74 (m, 2H, -CH_2), 3.15 (t, 2H, 5-CH_2-), 3.71 (t, 2H, Ar-CH_2-), 4.78 (t, 2H, 6-CH_2-), 6.16 (s, 2H, -OCH_2O-), 6.48 (s, 2H, -OCH_2O-), 7.10 (s, 1H, 4-Ar-H), 7.72 (s, 1H, -Ar-H), 7.83 (d, 1H, 11-Ar-H), 8.02 (d, 1H, 12-Ar-H), 8.79 (s, 1H, 13-Ar-H). ^{13}C NMR 13.52, 22.21, 26.50, 29.57, 31.57, 49.32, 101.98, 103.48, 105.68, 107.79, 113.39, 119.52, 120.27, 121.42, 122.70, 130.64, 131.98, 137.36, 144.11, 147.61, 148.52, 149.53, 160.11.

Compound 3: It was obtained as russet flakes from MeOH R_f 0.87, silica gel-GF_{254} C_6H_6/EtOAc/MeOH/iC_3H_7OH/NH_3 (6:3:1.5:1.5:0.5). ^{1}H NMR (300 MHz, DSMO-d_6): δ 0.93 (t, J = 6.8 Hz, 3H, -CH_3), 1.35 [m, 4H, -$(CH_2)_2$-], 1.55 (m, 2H, -CH_2), 1.76 (m, 2H -CH_2-), 3.16 (t, 2H, 5-CH_2-), 3.70 (t, 2H, Ar-CH_2-), 4.79 (t, 2H, 6-CH_2-), 6.17 (s, 2H, -OCH_2O-), 6.48 (s, 2H, -OCH_2O-), 7.10 (s, 1H, 4-Ar-H), 7.72 (s, 1H, -Ar-H), 7.84 (d, 1H, 11-Ar-H), 8.02 (d, 1H, 12-Ar-H), 8.80 (s, 1H, 13-Ar-H) ^{13}C NMR 13.95, 21.99, 26.56, 27.57, 28.72, 30.74, 31.90, 49.38, 102.03, 103.52, 105.72, 107.83, 113.44, 120.30, 120.36, 121.47, 122.77, 130.69, 132.04. 137.42, 144.15, 147.64, 147.68, 149.58, 160.13.

Compound 4: It was obtained as russet flakes from MeOH R_f 0.89, silica gel-GF_{254} C_6H_6/EtOAc/MeOH/iC_3H_7OH/NH_3 (6:3:1.5:1.5:0.5). ^{1}H NMR (300 MHz, DSMO-d_6): δ 0.86 (t, J = 6.8 Hz, 3H, -CH_3), 1.23 [m,4H,-$(CH_2)_4$-], 1.55 (m, 2H, -CH_2), 1.76 (m, 2H -CH_2-), 3.16 (t, 2H, 5-CH_2-), 3.70 (t, 2H, Ar-CH_2-), 4.79 (t, 2H, 6-CH_2-), 6.17 (s, 2H, -OCH_2O-), 6.48 (s, 2H, -OCH_2O-), 7.11 (s, 1H, 4-Ar-H), 7.73 (s, 1H, -Ar-H), 7.84 (d, 1H, 11-Ar-H), 8.03 (d, 1H, 12-Ar-H), 8.79 (s, 1H, 13-Ar-H). ^{13}C NMR 13.99, 22.12, 26.54, 26.78, 27.63, 28.55, 29.08, 29.61, 31.29, 31.88, 49.37, 102.03, 103.52, 105.72, 107.83, 113.44, 120.30, 120.36, 121.47, 122.77, 130.69, 132.04, 137.42, 144.15, 147.64, 147.68, 149.58, 160.13.

Compound 5: It was obtained as russet flakes from MeOH R_f 0.91, silica gel-GF_{254} C_6H_6/EtOAc/MeOH/iC_3H_7OH/NH_3 (6:3:1.5:1.5:0.5) ^{1}H NMR (300 MHz, DSMO-d_6): δ 0.86 (t, J = 6.8 Hz, 3H, -CH_3), 1.22 [m, 4H, -$(CH_2)_6$-], 1.54 (m, 2H, -CH_2), 1.75 (m, 2H -CH_2-), 3.14 (t, 2H, 5-CH_2-), 3.70 (t, 2H, Ar-CH_2-), 4.79 (t, 2H, 6-CH_2-), 6.17 (s, 2H, -OCH_2O-), 6.48 (s, 2H, -OCH_2O-), 7.11 (s, 1H, 4-Ar-H), 7.73 (s, 1H, -Ar-H), 7.85 (d, 1H, 11-Ar-H), 8.03 (d, 1H, 12-Ar-H), 8.81(s, 1H, 13-Ar-H) ^{13}C NMR 13.98, 22.13, 26.57, 27.65, 28.60, 28.74, 28.93, 29.05, 29.09, 31.33, 31.89, 49.40, 102.03, 103.51, 105.72, 107.82, 113.43, 120.28, 120.35, 121.44, 122.80, 130.65, 132.04, 137.39, 144.11, 147.61, 147.65, 149.57, 160.11.

Compound 6: It was obtained as russet flakes from MeOH R_f 0.94, silica gel-GF_{254} C_6H_6/EtOAc/MeOH/iC_3H_7OH/NH_3 (6:3:1.5:1.5:0.5) ^{1}H NMR (300 MHz, DSMO-d_6) 0.84 (t, J = 6.8 Hz, 3H, -CH_3), 1.23 [m, 8H, $(CH_2)_8$], 1.53 (m, 2H, -CH_2), 1.74 (m, 2H, -CH_2-), 3.14 (t, 2H, 5-CH_2-), 3.69 (t, 2H, Ar-CH_2-), 4.78 (t, 2H, 6-CH_2-), 6.16 (s, 2H, -OCH_2O-), 6.47 (s, 2H, -OCH_2O-), 7.09 (s, 1H, 4-Ar-H), 7.83 (s, 1H, -Ar-H), 7.98 (d, 1H, 11-Ar-H), 8.01 (d, 1H, 12-Ar-H), 8.79 (s, 1H, 13-Ar-H), ^{13}C NMR 13.98, 22.12, 26.55, 27.63, 28.59, 28.72, 28.93, 28.99, 29.06, 30.82, 31.32, 31.78, 31.88, 49.392, 102.03, 103.50, 105.73, 107.83, 113.45, 120.29, 120.35, 121.46, 122.79, 130.67, 132.04, 137.41, 144.14, 147.62, 147.66, 149.58, 160.14.

The spectral data of compounds **2-6** are shown that from the ^{1}H NMR, the H peak data of 9.91 position of coptisine disappeared and there is no H signal at above δ3 for coptisine, and the compounds **2-6** at 0.8-3.0 area have signal of H of alky, the number of H of alky is same to H of alky switching on at 8 position of coptisine. the compounds **2-6** at 13-55 area have increased the signal of c of alky, the increased number of c of alky is same to c of alky switching on at 8 position of coptisine. Hence, it is concluded that compounds **2-6** were synthesized successfully.

Antimicrobial activity: The compounds **1-6** were investigated *in vitro* for antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria and a fungus. Firstly, we used inhibition zone method to give preliminary evaluation to antibacterial effect of compounds of **1-6**, the result shows the antimicrobial activities of the compounds **2-6** were higher than that of coptisine.The minimum inhibitory concentration was evaluated by using the two-fold serial dilution test[14]. Compounds **1-6** were dissolved in H_2O containing 1 % DMSO-d_6 and diluted to different concentrations from 3.91 to 500 mg/mL with liquid medium. The mixtures of serious dilutions of compounds and the microbes (2×10^8 cfu/mL) in broth medium were incubated at 37 °C for 24 h for bacteria and at 25 °C for 48 h for the fungus. Microbial growth was examined by measuring the absorbance at 655 nm with a spectrophotometer[15]. The H_2O/DMSO-d_6 was used as a blank and berberine as positive control (Table-1)

TABLE-1
MINIMUM INHIBITORY CONCENTRATION OF COMPOUNDS 1-6 (ppm)

Compd.	Gram +ve bacteria		Gram –ve bacteria	Fungi	
	S. aureus	*B. subtilis*	*Proteus vulgaris*	*C. albicans*	*Dysenteriae*
Control	–	–	–	–	–
1	250	250	500	250	250
2	62.5	125	250	62.5	31.25
3	31.25	31.25	62.5	31.25	15.63
4	15.63	3.91	31.25	7.81	3.91
5	31.35	15.63	62.50	31.25	15.63
6	125	62.50	125	62.5	32.5
7	125	250	250	500	250

'–' show no values given, all experiments were run in triplicate. DMSO was used as blank and berberine as positive control, compound **7** is on behalf of berberine.

Test of LD_{50}: The LD_{50} was determined[16] to evaluate the toxicity of compounds **1-6** (Table-2). Healthy Kunming mice of both genders (20 ± 2 g, 7 weeks old) were purchased from the Laboratory Animal Centre of Chongqing Medical University. They were randomly divided into 36 groups with 10 mice each. Animal care and toxicity test procedure were carried out[16]. The given dosages of compounds **1-7** were designed according to Table-3. Approach to medicine:abdominal cavity injected. The normal control group was injected with the same volume of physiological saline. (1 % DMSO), mice were housed in stainless cages in a room with controlled temperature (23 ± 2 ºC) and humidity (40-60 %) and a 12 h light/dark cycle. The protocol complied with the guidelines of Chongqing City Laboratory Animal Administration Committee of China for the care and use of laboratory animals. Animals were then kept under observation for 7 days to record toxicity and total mortality.

TABLE-2
LD_{50} OF COPTISINE AND ITS DERIVIATES (mg/kg body weight)

Compd.	1	2	4	5	6	7
LD_{50}	95	25	50	65	80	50

Note: Compound **7** is berberine, berberine was used as control. Approach to medicine (Mice's abdominal cavity injected)

TABLE-3
DOSAGES OF COMPOUNDS **1-7**

Compd.	Dosage (mg/kg body weight)					
1,6	125	110	95	80	65	50
2,3	50	40	35	30	25	20
4,5,7	100	80	65	50	35	20

Compound **7** is berberine, approach to medicine (Mice's abdominal cavity injected)

RESULTS AND DISCUSSION

The 8-alkylcoptisine derivatives were synthesized successfully (**Scheme-I**). The yield of the derivatives decreased from 60 to 32 % as the aliphatic chain length increased. The spectral data of compounds **2-6** are shown in section 3, from the ^1H NMR data, the H peak data of 8 position of coptisine (δ 9.91, H-8) disappeared and there is no H signal at above δ3 and the compounds **2-6** at 0.8-3.0 area have signal of H of alky, the number of H of alky is same to H of alky switching on at 8 position of coptisine. Hence, it is concluded that compounds **2-6** were synthesized successfully and their structures are shown in Fig. 1. The antimicrobial activities of compounds **2-6** were 4-128 times higher than that of coptisine (**1**) and displayed more potency against fungi than towards bacteria (Table-1). The antimicrobial activity of compounds **2-4** increased as the aliphatic chain length increased and then gradually decreased (compounds **5** and **6**) when the number of carbon atoms was higher than eight. 8-Octyl-coptisine (**5**) showed the strongest activity against the microbes tested. LD_{50} of compounds **2-5** decreased as the aliphatic chain length increased and then gradually decreased.

It is concluded that by introducing the suitable alkyl groups to 8 position of coptisine can increase the antibacterial activity. The toxicity of compounds **2-6** was stonger than that of compound **1**, the longer the aliphatic chain of **2-6**, the lower was their toxicity, compound **6** showed almost the same low toxicity as coptisine.

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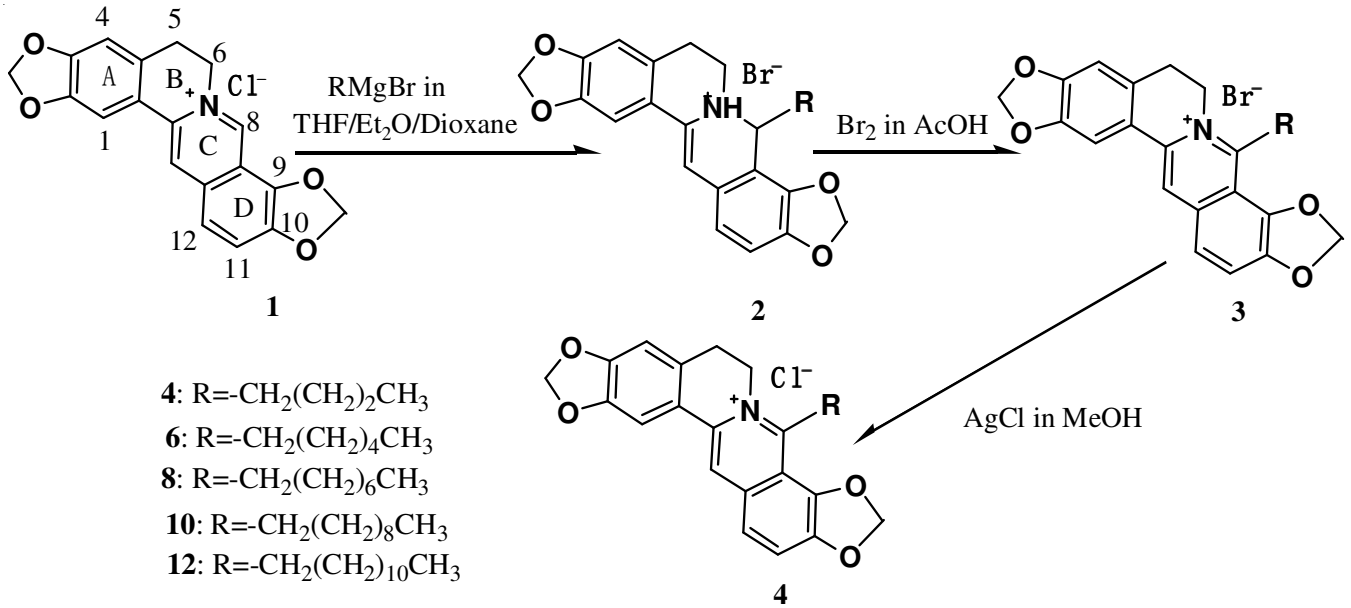

Scheme-I: Synthetic of 8-alkyl coptisine derivatives (compounds **2-6**)

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