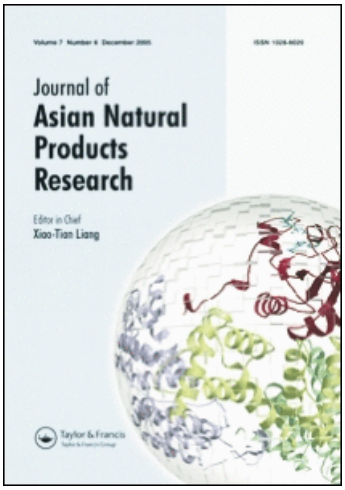

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Relationship between the antibacterial and immunological activities of houttuyfonate homologues and their surface activities

X. -L. Ye[a]; X. -G. Li[b]; L. -J. Yuan[c]; H. -M. He[b]
[a] School of Life Science, Southwest China Normal University, Chongqing, China [b] Chemistry Institute of Pharmaceutical Resources, Southwest Agricultural University, Chongqing, China [c] Inspection Centre for Agri-food Quality and Safety, Southwest Agricultural University, Chongqing, China

To cite this Article Ye, X. -L. , Li, X. -G. , Yuan, L. -J. and He, H. -M.(2006) 'Relationship between the antibacterial and immunological activities of houttuyfonate homologues and their surface activities', Journal of Asian Natural Products Research, 8: 4, 327 — 334
To link to this Article: DOI: 10.1080/10286020500035243
URL: http://dx.doi.org/10.1080/10286020500035243

Journal of Asian Natural Products Research, Vol. 8, No. 4, June 2006, 327–334

Relationship between the antibacterial and immunological activities of houttuyfonate homologues and their surface activities

X.-L. YE†, X.-G. LI‡*, L.-J. YUAN¶ and H.-M. HE‡

†School of Life Science, Southwest China Normal University, Chongqing 400715, China
‡Chemistry Institute of Pharmaceutical Resources, Southwest Agricultural University, Chongqing 400716, China
¶Inspection Centre for Agri-food Quality and Safety, Southwest Agricultural University, Chongqing 400716, China

(Received 17 June 2004; revised 29 September 2004; in final form 10 October 2004)

Based on the microbial model (*Staphylococcus aureus* and *Bacillus subtilis)* and the experiment of mouse carbon granular clearance, the relationship between antibacterial and immunological activities of houttuyfonate (HOU) homologues and their surface activities were studied. The results showed that with elongating the carbon chain of HOU homologues, the surface activities and bacteriostasis and immunological activities would be increased. It is suggested that the pharmacological effect of HOU homologues could be realized by the hydrophobic interaction between HOU homologues and membrane proteins of bacteria and cell.

Keywords: Houttuyfonate (HOU) homologues; Surface tension; Bacteriostasis; Immune activity

1. Introduction

Houttuynina cordata Thunb is used as a traditional Chinese herbal medicine, which can improve body immunity, clear away heat, eliminate carbuncles and purulence, and diminish inflammation [1]. Decanoyl acetaldehyde is a main antibacterial component in *Houttuynina cordata Thunb*, named houttuynine. However, the content of houttuynine in *Houttuynia cordata Thunb* is minimal, and its water-extracting rate is about 0.00049% [2]. In addition, houttuynine is insoluble in water, and unstable *in vitro* [3]. Therefore, houttuynine has been transformed into decanoyl acetal sodium sulfite, named houttuyninum, which has better water solubility and stability, and has antibacterial activity as good as houttuynine [4]. Subsequently, houttuyninum has been substituted for houttuynine in the clinical setting.

According to the molecular structure of houttuyninum, dodecanoyl acetal sodium sulfite has been synthesized and named sodium new houttuyfonate [5]. At present, houttuyninum

*Corresponding author. E-mail: xuegangli2000@yahoo.com.cn

Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2006 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020500035243

and sodium new houttuyfonate are used to treat respiratory system diseases, influenza, acute or chronic bronchitis and pneumonia, etc. [3,6].

In our laboratory, four kinds of houttuyfonate homologues ($C_nH_{2n+1}C(O)CH_2C(OH)SO_3$-Na, $n = 6,8,10,12$; abbreviated as HOU-C_n) were synthesized by changing the length of alkyl chain based on the structure of houttuyninum (HOU-C_{10}) [7].

HOU-C_{10} is a typical surfactant, which has a hydrophobic alkyl group and a hydrophilic vitriol group. Up to now, there is no report on the relationship between the antibacterial and immune activities of HOU-C_n and their surface activities. In our experiment, *Staphylococcus aureus* and *Bacillus subtilis* were used as the microbe model to study the antibacterial activity of HOU-C_n, and white mice were used as the animal model to investigate the effect of carbon chain length on immunological function. By investigating the relationship between the surface activity and biological activity of HOU-C_n, more potent and lower side-effect medicines might be obtained.

2. Results and discussion

2.1 Surface tension of HOU-C_n

According to their structure, HOU-C_n belong to surfactants. The surface tension of HOU-C_n versus logarithm of their concentration is shown in figure 1. According to the turning point of curves, critical micelle concentration (CMC) of HOU-C_n can be determined. Therefore, the CMCs of HOU-C_6, HOU-C_8, HOU-C_{10} and HOU-C_{12} were 5.32×10^{-3} mol/L, 1.41×10^{-3} mol/L, 3.88×10^{-4} mol/L and 1.06×10^{-4} mol/L, respectively.

Figure 2 shows that the carbon number of HOU-C_n versus logarithm of their CMC is a linear relationship. Therefore, the free energy of HOU-C_n forming micelle can be calculated based on its CMC, according to the formula as follows [8]:

$$\Delta G = 2.303 \times \mathrm{RT} \times \log(CMC)$$

The longer the carbon chain of HOU-C_n is, the smaller are the CMC and the ΔG. Correspondingly, the easier the micelle that would be formed and the stronger the hydrophobic

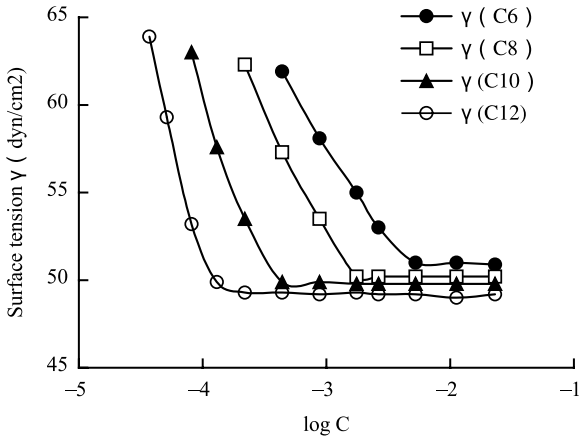

Figure 1. Surface tension versus logarithm of concentration of HOU-C_n.

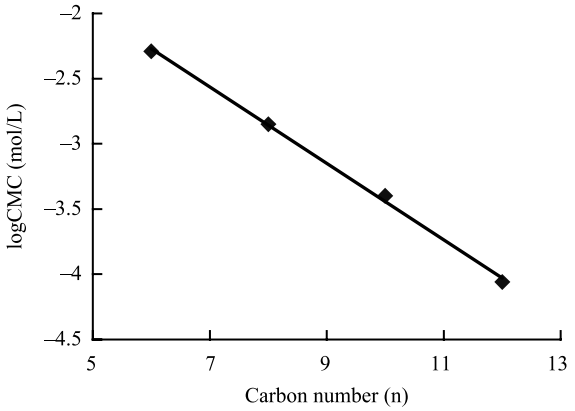

Figure 2. Relationship between carbon number of HOU-C_n and its logCMC

interaction that would exist with other hydrophobic groups. It was indicated that the hydrophobic ability increased along with elongation of the carbon chain.

2.2 Bacteriostasis of HOU-C_n to Staphylococcus aureus

The lower the absorbency of the media is, the more potent is the bacteriostatic effect of HOU-C_n. From figure 3, the bacteriostasis of HOU-C_n to *Staphylococcus aureus* would obviously increase along with the increase of HOU-C_n concentration, and the bacteriostatic effect is directly proportional to the carbon number of HOU-C_n. The longer the carbon chain of HOU-C_n is, the stronger are their bacteriostasis to *Staphylococcus aureus* and the lower are the concentrations of fully inhibiting *Staphylococcus aureus*. The full-inhibition concentration of HOU-C_6, HOU-C_8, HOU-C_{10} and HOU-C_{12} was 0.553, 0.363, 0.226 and 0.153 mg/ml, respectively (figure 3).

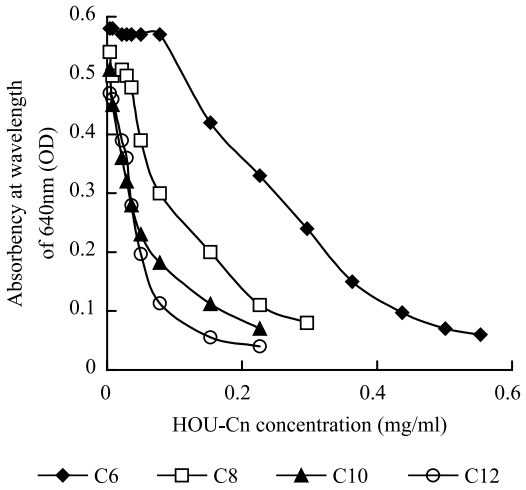

Figure 3. Bacterioostasis of HOU-C_n to *Staphylococcus aureus*.

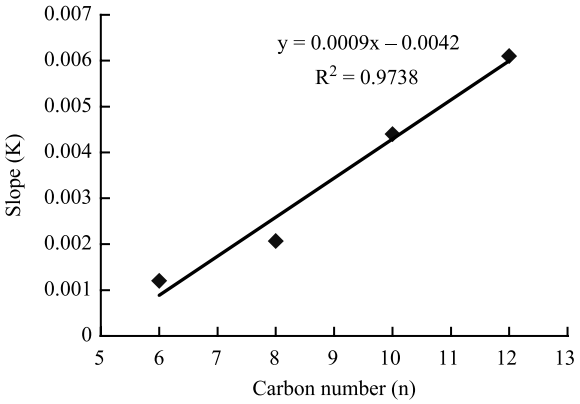

Figure 4. Carbon number of HOU-C_n versus bacteriostatic activity to *Staphylococcus aureus*.

From figure 4, the slope of precipitous curves versus carbon number of HOU-C_n indicated that the slope of curve would be increased in parallel with the increase of carbon number of HOU-C_n. This indicates that bacteriostasis of HOU-C_n to *Staphylococcus aureus* was positively proportional to the carbon number of HOU-C_n. In these four kinds of HOU-C_n, the bacteriostatic activity of HOU-C_{12} was the strongest.

2.3 Bacteriostasis of HOU-C_n to Bacillus subtilis

Figure 5 shows the bacteriostasis of HOU-C_n to *Bacillus subtilis*. The bacteriostasis to *Bacillus subtilis* was similar to that to *Staphylococcus aureus* (shown in figure 3). Within a certain concentration, bacteriostasis of HOU-C_n would be strengthened along with the concentration increase, and bacteriostasis of HOU-C_n would also be strengthened alongside elongation of the carbon chain.

Figure 6 shows that the relationship between the slope of the precipitous curve was directly proportional to the carbon number of HOU-C_n. It indicates that the longer the carbon number

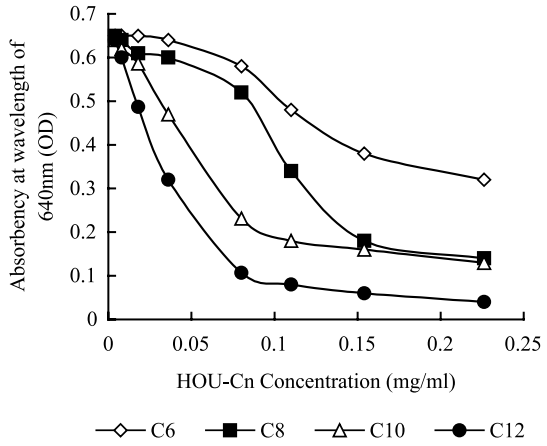

Figure 5. Bacteriostasis of HOU-C_n to *Bacillus subtilis*.

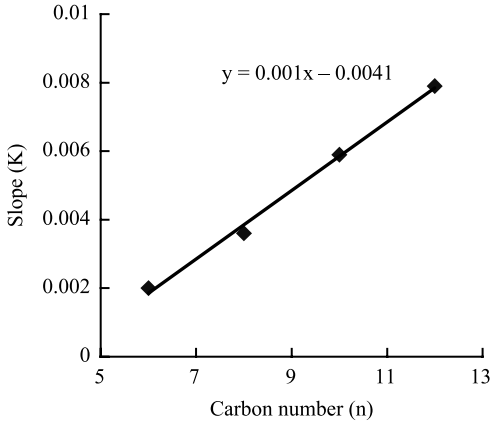

Figure 6. Carbon number of HOU-C_n versus bacteriostatic activity to *Bacillus subtilis*.

of HOU-C_n is, the more potent is the bacteriostasis of HOU-C_n to *Bacillus subtilis*. Similarly, the bacteriostatic activity of HOU-C_{12} was the highest.

These results showed that hydrophobic ability and bacteriostasis of HOU-C_n would be increased along with an elongating carbon chain, and that bacteriostasis of HOU-C_n to *Staphylococcus aureus* and *Bacillus subtilis* was positively proportional to their hydrophobic ability.

2.4 Determination of immune activity of HOU-C_n

Table 1 shows that after injecting 3 mg/kg of HOU-C_n into mice for 7 days, HOU-C_n could increase the carbon granular clearance rate *K* of macrophage, especially HOU-C_{12}, and that the phagocytic function of macrophages in mice injected with HOU-C_n was enhanced. There was little influence of HOU-C_n on thymus gland index and spleen index, suggesting that HOU-C_n could improve the immunological function of mice.

The effects of different concentrations of HOU-C_n on immune ability are shown in figure 7. Under the same dose, immune activity of mice rose steadily alongside the elongation of the carbon chain. In the dose range of 1.5–6 mg/kg of HOU-C_n, immune ability increased along with the rise of the dose. The results differed from literature reports, which found that when the dose was higher than 6 mg/kg, the immune function of mice was enhanced along with a decrease of the dose of HOU-C_{10} [6].

Table 1. Effects of sodium houttuyfonate homologues on the immune organs of mice ($\bar{x} \pm s$).

Group	*Carbon granular clearance rate K*	*Thymus gland index (mg/g)*	*Spleen index (mg/g)*
CK	0.0129 ± 0.0031	1.3997 ± 0.1223	3.9762 ± 0.1513
C_6	0.0197 ± 0.0033*	1.3818 ± 0.1369	4.0175 ± 0.1272
C_8	0.0241 ± 0.0038*	1.4046 ± 0.1121	4.0322 ± 0.1315
C_{10}	0.0286 ± 0.0037*	1.3802 ± 0.1215	4.0279 ± 0.1411
C_{12}	0.0330 ± 0.0155**	1.4051 ± 0.1198	4.0772 ± 0.1331

Dose of HOU-C_n is 3 mg/kg. *$p < 0.05$, **$p < 0.01$ compared with CK.

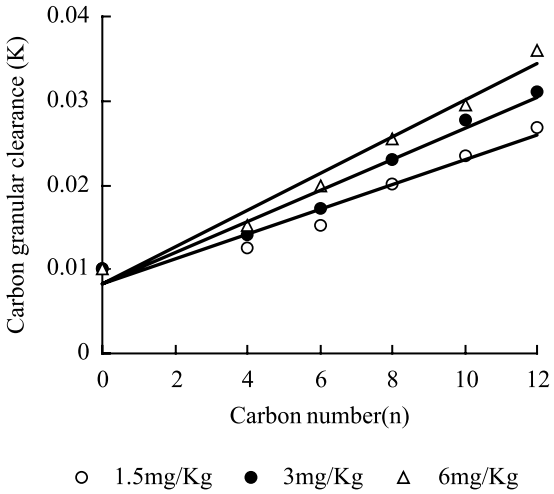

Figure 7. Carbon number versus immune activity of HOU-C_n.

Our results showed that improved immunity of HOU-C_n was positively proportional to the length of the carbon chain, and that the biological activity of HOU-C_n was directly proportional to its hydrophobic ability.

2.5 Discussion

The results of microbial and animal experiments showed that the bacteriostasis and immune function of HOU-C_n were proportional to the length of their carbon chain. A surface tension test indicated that the hydrophobic activity of HOU-C_n was directly proportional to their carbon chain length.

Previous studies [8] showed that a surfactant would bind with the hydrophobic site of protein or enzyme, causing the conformation and biological activity of the protein to be changed. Further evidence reported [9] that the longer the carbon chain of the surfactant, the stronger was the ability of their denaturalization to enzyme and protein. HOU-C_n was a typical surfactant, whose bacteriostasis and immune activities were positively proportional to their hydrophobic activity, which was similar to that of surfactant and enzyme [8]. This suggested that the pharmacological effect of HOU-C_n could be caused by the hydrophobic combination between HOU-C_n and the membrane protein of bacteria and cell.

The stronger the hydrophobic activity of HOU-C_n is, the better the immune and antibacterial activity of HOU-C_n are. It is speculated that HOU-C_n could combine with the hydrophobic site of membrane protein by hydrophobic interaction, because the stronger hydrophobic activity of HOU-C_n would be more beneficial if combined with bacteria and cell, further improving physiological activity.

Experimental results showed that in the dose range of 1.5–6 mg/kg of HOU-C_n, immune ability was increased alongside the rise of the dose. But when the dose was too high, the immune function of mice decreased in parallel with the increase of the dose of HOU-C_{10} [6]. Within a range of a certain concentration, a surfactant would make the conformation of

protein more flexible and increase enzyme activity. However, when its concentration was too high, it would make the conformation of protein change to excess and further cause enzyme activity to be lost.

3. Experimental

3.1 Synthesis of sodium houttuyfonate homologues

Sodium houttuyfonate homologues ($C_nH_{2n+1}C(O)CH_2C(OH)SO_3Na$, abbreviated as HOU-$C_n$) were synthesized according to Li *et al*. [7], including hexanoyl acetal sodium sulfite (HOU-C_6), octanoyl acetal sodium sulfite (HOU-C_8), decanoyl acetal sodium sulfite (HOU-C_{10}) and dodecanoyl acetal sodium sulfite (HOU-C_{12}). All samples in the experiments were recrystallised five times, and their contents were more than 97% analysed according to the standard methods of the Chinese Pharmacopoeia.

3.2 Determination of surface tension [9]

The concentrations of HOU-C_n were controlled at 0.025 mol/L. HOU-C_n decompose in water and release sodium hydrogen sulphite. When measuring their surface tension, 0.5 mol/L of sodium hydrogen sulphite should be added into the above solutions to keep these compounds stable. The water solubility of HOU-C_{10} and HOU-C_{12} was very low, therefore the temperature of the solution should be controlled at 55 ± 0.5°C when measuring their surface tension.

3.3 Bacteriostasis of HOU-C_n [10]

Staphylococcus aureus and *Bacillus subtilis* were grown on 5 ml of liquid media (pH 7.2) for about 6 h each, and adjusted to $10^5 - 10^6$ bacteria per milliliter under the microscope. HOU-C_n was diluted into different concentration solutions with liquid media. Liquid bacteria incubated for 6 h were planted into these HOU-C_n media. After being incubated for another 24 h, the absorbency of the above media was determined at 640 nm by a spectrophotometer, then curves of HOU-C_n versus bacteriostasis were calculated in which HOU-C_n concentration was the abscissa and absorbency was the ordinate. The beginning bacteriostatic concentration was taken as that one just as absorbency of the media decreased, and the full inhibition concentration when absorbency of the media was equal to that of the control. Fifty microlitres of HOU-C_n media were taken from the test tube in which bacteria had been fully inhibited and planted in the plate media, then incubated for about 24 h. The bactericidal concentration was determined if there had been no bacteria in the plate media.

3.4 Immune activity of HOU-C_n

Healthy mice (18–22 g) were randomly divided into 13 groups, each containing 10 mice. Therapy groups included HOU-C_n. Every kind of HOU-C_n was divided into three gradient groups, according to concentrations of 0.1, 0.2 or 0.4 mg/ml injections of HOU-C_n.

In therapy groups, every mouse was injected with 0.3 ml of HOU-C_n and the control group was injected with the same dose of physiological saline. In fact, the dose of HOU-C_n injected into mice every time was 1.5, 3 or 6 mg/kg. Every mouse was injected for 7 days. The day after the last injection, every mouse was weighed, and then injected in the tail vein with diluted India ink at a dose of 0.1 ml/10 g·body weight. Blood (20 μl) was taken with a capillary at 3 and 10 min after injection respectively, and dissolved in 1.5 mL of 0.1% Na_2CO_3 and shaken up evenly. Absorbency of the above solution was measured at the wavelength of 680 nm.

After taking blood, the mice were killed humanely and the liver, spleen and thymus gland taken out and weighed. Immune ability was determined according to the carbon granular clearance rate (K) [6], revised index, thymus gland index and spleen index.

The Carbon granular clearance rate K was calculated by following equation:

$$K = \frac{\log A_1 - \log A_2}{t_2 - t_1}$$

3.5 Statistical analysis

Experimental data were analysed by DPS statistical software.

References

[1] S. Li, Q.H. Yu, Z.Y. Chu. *Chin. Pharm. Bull.*, **14**, 442 (1998).
[2] X.Y. Hao, L. Li, Z.H. Ding. *Acta Bot. Yunnan.*, **17**, 350 (1995).
[3] S.C. Wei, L.J. Xu, Q. Zeng. *Chin. Pharm. J.*, **34**, 167 (1999).
[4] L. Shao, Q.H. Yu, Q. Huang, S.H.S. Liu. *Chin. Pharm. Bull.*, **17**, 51 (2001).
[5] C.H.Q. Yuan. *Exploitation and Utilization of Natural Medicine Resource*, pp. 1569–1575, Jiangsu Science and Technique Public House, China (2000).
[6] A.F. Du, S.H. Hu, W.H. Bao. *J. Trad. Chin. Vet. Med.*, **17**, 7 (1998).
[7] X.G. Li, L.J. Yuan, H.M. He. Advanced water-soluble houttuynine. *China invention patent* application No. 2004100044932 (2004).
[8] X.G. Li. *Acta Biophys. Sin.*, **11**, 13 (1995).
[9] X.G. Li. *Physical Chemistry of Surfactants and their Application on Agriculture*, pp. 52–70, Southwest China Normal University Public House, China (1997).
[10] H. Li, H.C. Jiang, G.L. Zhou. *Acta Botanica Yunnanica*, **24**, 95 (2002).