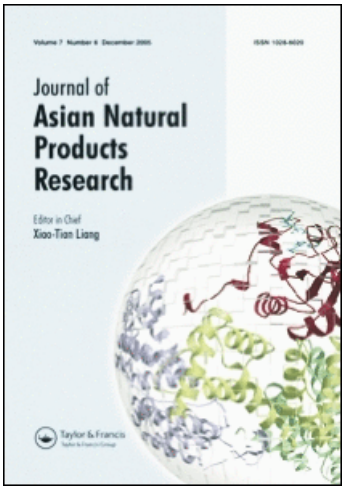

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Effect of octadecanoyl acetal sodium sulphite series with different numbers of double bonds on their immunological and surface activities

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

To cite this Article Yuan, L. -J. , Ye, X. -L. , Li, X. -G. and He, H. -M.(2007) 'Effect of octadecanoyl acetal sodium sulphite series with different numbers of double bonds on their immunological and surface activities', Journal of Asian Natural Products Research, 9: 1, 49 — 58
To link to this Article: DOI: 10.1080/10286020500289659
URL: http://dx.doi.org/10.1080/10286020500289659

Journal of Asian Natural Products Research, Vol. 9, No. 1, January–February 2007, 49–58

Effect of octadecanoyl acetal sodium sulphite series with different numbers of double bonds on their immunological and surface activities

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

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

(Received 26 January 2005; revised 23 March 2005; in final form 25 June 2005)

The relationship between the immunological activities and their surface activities of octadecanoyl acetal sodium sulphite series containing different numbers of double bonds (HOU-$C_{18:n}$; $n = 1$, Δ^9; $n = 2$, $\Delta^{9,12}$; $n = 3$, $\Delta^{9,12,15}$) were studied. The results showed that HOU-$C_{18:n}$ were able to increase the carbon granular clearance rate K, the NK cell activity in spleen and the activity of lysozymes in serum as well as inhibit *Staphylococcus aureus* and lysozymes *in vitro* to some degree. As the number of double bonds in HOU-$C_{18:n}$ increased along with the hydrophobic properties and the ability to improve the immune activity, NK cell activity and lysozyme activity decreased, but the bacteriostatic activity increased. It is speculated that HOU-$C_{18:n}$ could improve immunity and bacteriostasis realised by the interaction between the hydrophobic chain and membranes of cells. From the results of the effects of HOU-$C_{18:n}$ on lysozymes *in vivo* and *in vitro*, it is speculated that HOU-$C_{18:0}$ could initiate and greatly enhance lysozyme activity in serum by increasing the amount of lysozymes, while unsaturated HOU-$C_{18:n}$ might do so mainly by increasing the number of lysozymes.

Keywords: Octadecanoyl acetal sodium sulphite series (HOU-$C_{18:n}$); Surface tension; Bacteriostasis; Immunological activity

1. Introduction

Houttuynina cordata Thunb is used as a traditional Chinese herbal medicine, which is used to improve body immunity, as an antipyretic, to eliminate carbuncle and purulence, and diminish inflammation [1]. Decanoyl acetaldehyde is a main antibacterial component in *Houttuynina cordata Thunb*, named houttuynine. Previous studies showed that houttuyfonate homologues ($C_nH_{2n+1}C(O)CH_2C(OH)SO_3Na$, $n = 6$, 8, 10, 12; abbreviated as HOU-C_n) improved the immune ability of mice and inhibited the growth of *Staphylococcus aureus* and *Bacillus subtilis*, and that the elongating the carbon chain of HOU-C_n ($n = 4$, 6, 8, 10, 12) could steadily increase the surface activities and bacteriostasis, and immunological activity [2].

*Corresponding author. Email: xuegangli2000@yahoo.com.cn

Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2007 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020500289659

In fact, the alkyl chain in HOU-C_n comes from fatty acids. Many studies reported that free unsaturated fatty acid esters, such as linolenic acid, linoleic acid, oleic acid and arachidonic acid, had many important physiological functions, concerned with adjusting immunity ability, and forming and dissolving thrombus [3]. Polyunsaturated fatty acid (PUFA) inhibited the growth of gastric cancer cells by reducing cell proliferation and inducing apoptosis. These effects may be associated with the increase of lipid peroxidation production [4]. PUFA had cytotoxic effects on ascites tumour cell, and these effects were stronger than those of saturated fatty acid esters, comparing the same length of carbon chain [5]. PUFA was able to kill human carcinoma cells selectively, but had hardly any effect on normal cells [6]. Moderate PUFA improved immunity function. However, when the concentration of PUFA was too low or too high, it would inhibit immunity function and as the number of double bonds increased, the effect of PFUA would become more obvious [7,8].

Applying knowledge of the hydrophobic activity of HOU-C_n and the properties of double bonding in unsaturated fatty acids, new houttuyfonate analogues were synthesised by prolonging the carbon chain and by introducing unsaturated bonds to improve its pharmacological effect. At present, the octadecanoyl acetal sodium sulphite series with different numbers of double bonds (abbreviated as HOU-$C_{18:n}$, n indicating the number of double bonds, $n = 0, 1, 2, 3$) were synthesised by changing the numbers of double bonds in our experiment [9].

HOU-$C_{18:n}$ belongs to a new group of synthesised chemical compounds. Up to now, there has been no report on the relationship between the immune activity of HOU-$C_{18:n}$ compounds and their surface activity. In the present experiment, the effects of double bond numbers on immunological function, NK cell activity and lysozyme activity *in vivo* and *in vitro* were investigated. By examining the relationship between the surface activity and immunological activity of HOU-$C_{18:n}$, compounds with more potent activities could be obtained.

2. Results and discussion

2.1 Surface tension of HOU-$C_{18:n}$

The relationship of surface tension of HOU-$C_{18:n}$ versus logarithm of the concentration is shown in figure 1. According to the turning point of the curves, the critical micelle concentration (CMC) of HOU-$C_{18:n}$ was determined, shown in table 1.

The relationship between the numbers of double bonds of HOU-$C_{18:n}$ and logarithm of their CMC is shown in figure 2. These results indicated that they was a linear relationship. The free energy of HOU-$C_{18:n}$ forming micelle was calculated based on its CMC, according to the formula as follows [10]:

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

The less the numbers of double bonds in HOU-$C_{18:n}$, the lower the CMC and the ΔG are. Correspondingly, the easier the micelle would thus be formed and the stronger the hydrophobic interaction would be with other hydrophobic groups. These data indicated that the hydrophobic ability declined along with the increasing number of double bonds in HOU-$C_{18:n}$.

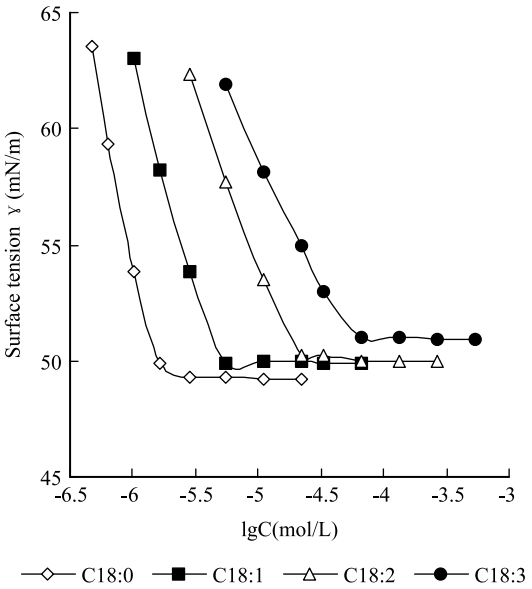

Figure 1. Relationship between IgC of HOU-$C_{18:n}$ with different double bond numbers and their surface tensions.

Table 1. The CMC and ΔG of Hou-$C_{18:n}$.

	HOU-$C_{18:0}$	*HOU-$C_{18:1}$*	*HOU-$C_{18:2}$*	*HOU-$C_{18:3}$*
CMC (mol L^{-1})	1.63×10^{-6}	4.89×10^{-6}	1.95×10^{-5}	5.13×10^{-5}
ΔG (kJ mol^{-1})	−36.35	−33.34	−29.58	−26.94

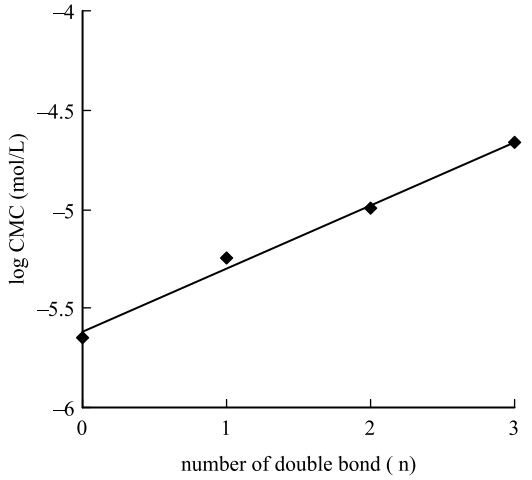

Figure 2. Relationship between double bond numbers and logCMC.

Table 2. Effects of HOU-$C_{18:n}$ on the immunity organs of mice ($\bar{x} \pm s$).

Groups	*Carbon granular clearance rate K*	*Thymus gland index (mg g^{-1})*	*Spleen index (mg g^{-1})*
Control	0.0155 ± 0.0082	1.3987 ± 0.1233	3.9971 ± 0.1323
HOU-$C_{18:0}$	0.0383 ± 0.0092**	1.3818 ± 0.1169	3.9754 ± 0.1311
HOU-$C_{18:1}$	0.0323 ± 0.0082**	1.3935 ± 0.1011	3.9912 ± 0.1351
HOU-$C_{18:2}$	0.0279 ± 0.0098*	1.3912 ± 0.1294	4.0165 ± 0.1390
HOU-$C_{18:3}$	0.0224 ± 0.0058*	1.4015 ± 0.1098	3.9983 ± 0.1382

The dose of HOU-$C_{18:n}$ is 3 mg kg^{-1}. *$P < 0.05$, **$P < 0.01$ compared with the control.

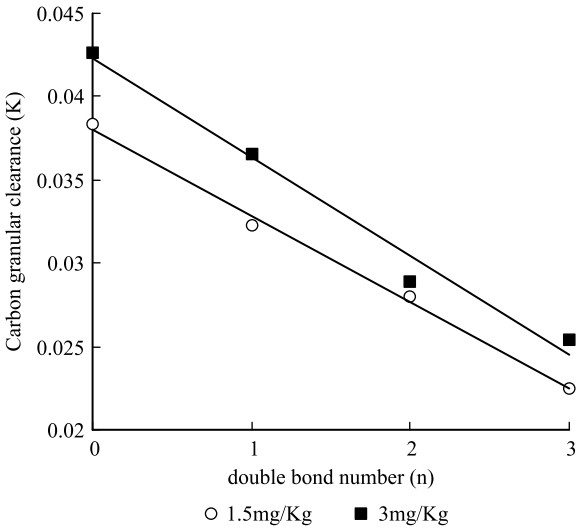

Figure 3. Double bond number versus immune activity of HOU-$C_{18:n}$.

2.2 Determination of immunity activity of HOU-$C_{18:n}$

Table 2 shows that after injecting 3 mg kg^{-1} of HOU-$C_{18:n}$ in mice for 7 days, HOU-$C_{18:n}$, especially HOU-$C_{18:0}$, was able to increase the carbon granular clearance rate *K* of macrophages. This indicated that phagocytic function of macrophages in mice being injected with HOU-$C_{18:n}$ would be strengthened, which would be more enhanced as the number of double bonds decreased. However, there was little influence of HOU-$C_{18:n}$ on thymus gland index and spleen index, suggesting that HOU-$C_{18:n}$ would improve the immunological function of mice.

Effects of different concentrations of HOU-$C_{18:n}$ on immunity ability are shown in figure 3. Under the same dose, immunity activity of mice declined steadily with the increase of double bond number. In the dose range of 1.5–3 mg kg^{-1} of HOU-$C_{18:n}$, immunity ability increased along with the rising dose, which is similar to previous results [2].

Results showed that improving immunity of HOU-$C_{18:n}$ was negatively proportional to the double bond number. However, the immunity activity of HOU-$C_{18:n}$ was directly proportional to its hydrophobic ability.

2.3 Effect of HOU-$C_{18:n}$ on the NK cell activity

Natural killer (NK) cells can kill various tumour cells or other cells infected with viruses, and also adjust immunity. Table 3 shows that HOU-$C_{18:n}$ increased the NK cell activity in spleen of normal mice to different degrees. HOU-$C_{18:0}$ increased the NK cell activity of mice

Table 3. Effects of HOU-$C_{18:n}$ on the NK cell activity from spleen of mice ($\bar{x} \pm s$).

	NK cell activity (%, $\bar{x} \pm s$)	
Groups	*1:25*	*1:50*
Control	29.2 ± 2.6	39.9 ± 2.9
HOU-$C_{18:0}$	40.8 ± 2.5**	53.8 ± 1.7**
HOU-$C_{18:1}$	37.7 ± 1.5*	49.8 ± 1.7*
HOU-$C_{18:2}$	33.6 ± 2.9	43.7 ± 2.3
HOU-$C_{18:3}$	30.6 ± 1.9	41.8 ± 2.6

The dose of HOU-$C_{18:n}$ is 3 mg kg^{-1}. *$P < 0.05$, **$P < 0.01$ compared with the control.

by about 40% compared with the control. However, as the number of double bonds increased, the NK cell activity declined. There was little influence of HOU-$C_{18:3}$ on NK cell activity. According to table 3 and figure 2, the degree of increasing NK cell activity by HOU-$C_{18:n}$ was directly proportional to their hydrophobic ability.

2.4 Effect of HOU-$C_{18:n}$ on the activity of lysozyme in serum of mice

According to figure 4, 1.5 mg kg^{-1} and 3 mg kg^{-1} HOU-$C_{18:n}$ activated lysozymes in serum of mice after injection. The activated degree of lysozyme activity in serum was lowered as the number of double bonds increased. HOU-$C_{18:0}$ increased the activity of lysozymes in serum by 60%. It is speculated that HOU-$C_{18:n}$ could activate the activity of lysozymes in serum by increasing the number of lysozymes in blood or by improving the catalysing efficiency of lysozymes.

2.5 Effect of HOU-$C_{18:n}$ on the activity of lysozymes in vitro

Figure 5 shows that HOU-$C_{18:0}$ increased the activity of lysozymes when its concentration was lower than 10 mg L^{-1}. Only when its concentration was more than 10 mg L^{-1}, the activity of lysozyme began to decrease, and then to be inhibited when its concentration rose to 100 mg L^{-1}. In general, in the range of low concentration of HOU-$C_{18:0}$, the activity of lysozymes *in vitro* would increase along with the rising concentration of HOU-$C_{18:0}$.

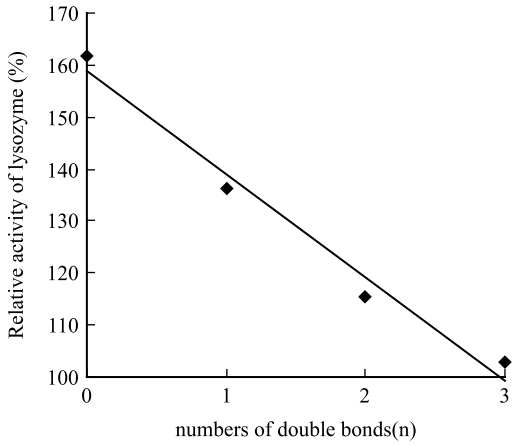

Figure 4. Effects of $C_{18:n}$ on the activity of lysozyme *in vivo*.

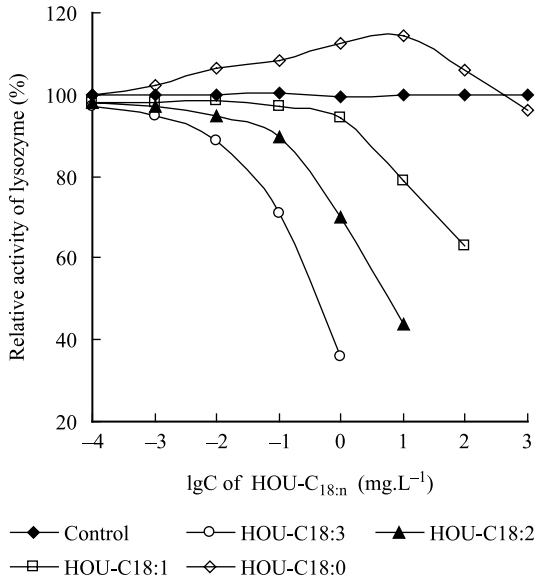

Figure 5. Effects of HOU-$C_{18:n}$ on the activity of lysozyme *in vitro*.

However, unsaturated HOU-$C_{18:n}$ could inhibit the activity of lysozymes in the concentrations tested. When the concentrations of HOU-$C_{18:3}$, HOU-$C_{18:2}$ and HOU-$C_{18:1}$ were more than 0.01, 0.2 and 4 mg L^{-1}, respectively, the activity of lysozymes weakened quickly, indicating that the inhibitory ability of HOU-$C_{18:n}$ would be increased along with the increasing double bond number.

2.6 Bacteriostasis of HOU-$C_{18:n}$ to Staphylococcus aureus

The lower the absorbency of the media is, the more potent is the bacteriostatic effect of HOU-$C_{18:n}$. As shown in figure 6, the bacteriostasis of HOU-$C_{18:n}$ to *Staphylococcus aureus* obviously increases along with the increase in HOU-$C_{18:n}$ concentration (300–700 mg L^{-1}), and the

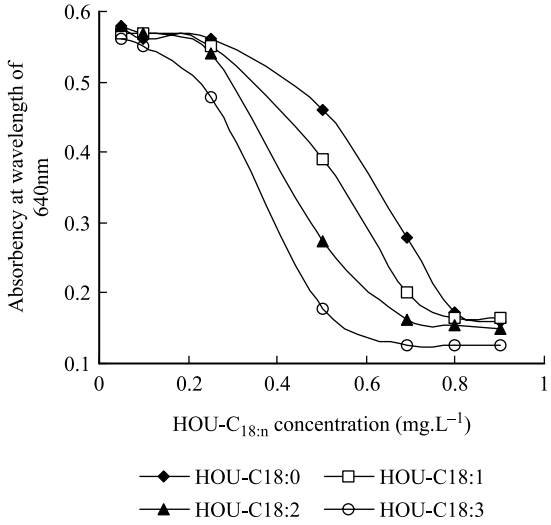

Figure 6. Bacteriostasis of HOU-$C_{18:n}$ to *Staphylococcus aureus*.

bacteriostatic effect is directly proportional to the number of double bonds in HOU-$C_{18:n}$. The more double bonds there are in HOU-$C_{18:n}$, the stronger is the bacteriostasis to *S. aureus* is and the lower is the concentration of fully inhibiting *S. aureus* is. The full-inhibition concentrations of HOU-$C_{18:0}$, HOU-$C_{18:1}$, HOU-$C_{18:2}$ and HOU-$C_{18:3}$ are 805, 710, 620 and 550 mg L^{-1}, respectively (figure 6). This indicated that bacteriostasis of HOU-$C_{18:n}$ to *S. aureus* was positively proportional to the number of double bonds in HOU-$C_{18:n}$. In these four kinds of HOU-$C_{18:n}$, the bacteriostatic activity of HOU-$C_{18:3}$ was the strongest.

2.7 Discussion

The results showed that HOU-$C_{18:n}$ was able to increase the carbon granular clearance rate *K*, the NK cell activity in spleen and the activity of lysozyme in serum, and inhibited *S. aureus* and lysozyme *in vitro* to some degree. Along with increasing the numbers of double bonds in HOU-$C_{18:n}$, the hydrophobic ability and the degree of improvement in the immune activity, the NK cell activity and lysozyme activity would be decreased, but the bacteriostasis activity would be increased.

In the currently known concept, cell membrane or bacteria biofilm is a bimolecular lipid layer, a semipermeable membrane. Because of their hydrophobic nature, the hydrocarbon chains in lipid bilayers provide a virtually impenetrable barrier to ionic and polar substances. Specific membrane proteins regulate the movement of such substances into and out of cells [11]. Studies have shown that fat-soluble substances simply diffuse through the lipid bilayer down their concentration gradients. The stronger the hydrophobic ability of a substance, the easier it diffuses through membranes [12].

Regarding the structure of HOU-$C_{18:n}$, they have a hydrophilic group and a hydrophobic alkyl chain. In general, HOU-$C_{18:n}$ with a longer alkyl chain would diffuse into cells easily and exert a pharmacological effect on bacteria or lysozymes. Studies showed that there were special proteins that could recognise some special groups in macrophage membrane [13], such as advanced glycosylation end product (AGE receptor, being the product of protein, HbAIC and glucose), a protein that can bind with the aldehyde group and further be modified by aldehyde. Modified protein can accelerate the moving of monocytes and foam cells, and increase the permeability. When HOU-$C_{18:n}$ is injected into mice, its aldehyde is exposed and can bind with some special protein sites on macrophage membrane. At the same time, HOU-$C_{18:n}$ can bind with the membrane by hydrophobic interaction. Once HOU-$C_{18:n}$ binds with the membrane, macrophages are stimulated and produce many stressed proteins or enzymes within it. On the other hand, its long hydrophobic chain might be infused through the membrane. Consequently, the concentration of HOU-$C_{18:n}$ in macrophage can be greatly increased.

The present results show that the hydrophobic ability of HOU-$C_{18:n}$ decreases as the number of double bonds increases. It is speculated that adsorption capacity on the membrane will be decreased correspondingly, and stimulated macrophages will decline in number. Therefore, the ability to improve the immune activity of HOU-$C_{18:n}$ will be decreased.

HOU-$C_{18:n}$ is a typical surfactant. Previous studies [14] showed that surfactant binds with the hydrophobic site of protein or enzyme, causing a change in conformation and biological activity of protein. Further evidence [10] showed that the stronger was the hydrophobic ability of surfactant, the stronger was the ability to adjust the conformation of enzyme and protein. Experiments *in vitro* showed that HOU-$C_{18:0}$ activates lysozymes while unsaturated

HOU-$C_{18:n}$ inhibits lysozymes. It was speculated that HOU-$C_{18:0}$ would enhance lysozyme activity by increasing the number of lysozymes and by initiating the activity of lysozymes, whereas unsaturated HOU-$C_{18:n}$ would enhance the activity of lysozymes by mainly increasing the number of lysozymes.

Our experimental results showed that the bacterisostasis of HOU-$C_{18:n}$ increased as the number of double bonds increased. Studies reported that unsaturated fatty acid could kill cancer cells by destroying the membrane [5,15]. It is speculated that the unsaturated bond of HOU-$C_{18:n}$ could destroy the membrane of bacteria and kill the bacteria. Although it was not too obvious that unsaturated HOU-$C_{18:n}$ improved the immune activity, it promises to provide a new kind of clinical approach towards curing cancer.

3. Experimental

3.1 Synthesis of octadecanoyl acetal sodium sulphite

Four kinds of octadecanoyl acetal sodium sulphite ($C_{17}H_{35-2n}C(O)CH_2C(OH)SO_3Na$, abbreviated as HOU-$C_{18:n}$, $n = 0, 1, 2, 3$) were synthesised according to the literature [8], including octadecanoyl acetal sodium sulphite ($CH_3(CH_2)_{16}C(O)CH_2C(OH)SO_3Na$, abbreviated as HOU-$C_{18:0}$), oleoyl acetal sodium sulphite ($CH_3(CH_2)_7CH = CH(CH_2)_7$-$C(O)CH_2C(OH)SO_3Na$, abbreviated as HOU-$C_{18:1}$, Δ^9), linoleoyl acetal sodium sulphite ($CH_3(CH_2)_4CH = CHCH_2CH = CH(CH_2)_7C(O)CH_2C(OH)SO_3Na$, abbreviated as HOU-$C_{18:2}$, $\Delta^{9,12}$) and linolenoyl acetal sodium sulphite ($CH_3CH_2CH = CHCH_2CH = CHCH_2$-$CH = CH(CH_2)_7C(O)CH_2C(OH)SO_3Na$, abbreviated as HOU-$C_{18:3}$, $\Delta^{9,12,15}$). All samples in the experiment 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

The concentrations of HOU-$C_{18:n}$ were controlled at 2.5×10^{-2} mol L^{-1}. As HOU-$C_{18:n}$ would decompose and release sodium hydrogen sulphite when dissolved in water, 0.5 mol L^{-1} of sodium hydrogen sulphite was added to the solutions to make them stable.

The water solubility of HOU-$C_{18:n}$ is very low, therefore the temperature of solution should be controlled at 55 ± 0.5°C when measuring the surface tension. The drop volume method is used to measure the surface tension of the solution [2,10].

3.3 Immune activity of HOU-$C_{18:n}$

Healthy mice of body weight 18–22 g were randomly divided into nine groups. Every 10 mice comprised one group. Therapy groups included HOU-$C_{18:n}$. Each type of HOU-$C_{18:n}$ was divided into two gradient groups. In therapy groups, every mouse was subcutaneously injected with 0.3 ml of HOU-$C_{18:n}$ (100 mg L^{-1} or 200 mg L^{-1}) and the control group was injected with the same dose of physiological saline. In fact, the injected dose of HOU-$C_{18:n}$ into mice each time was 1.5 or 3 mg kg^{-1}. The carbon granular clearance rate (K), thymus gland index and spleen index were determined according to a previous report [2].

3.4 Measurement of the natural killer cell (NK) activity in spleen of mice

Healthy mice (18–22 g) were randomly divided into five groups (HOU-$C_{18:0}$, HOU-$C_{18:1}$, HOU-$C_{18:2}$, HOU-$C_{18:3}$ and the control group). In therapy groups, every mouse was

subcutaneously injected with 0.3 ml of HOU-$C_{18:n}$ at a concentration of 200 mg L^{-1}, and the control group was injected with the same dose of physiological saline then continued for 7 days. The actual dose of HOU-$C_{18:n}$ was about 3 mg kg^{-1}. The next day the mouse was killed, and its spleen taken out and placed into Hanks' solution. The NK cell activity was measured according to the method reported previously [16].

3.5 Determination of lysozyme activity in serum of mice

3.5.1 Separation of serum. Healthy mice (18–22 g) were randomly divided into five groups (HOU-$C_{18:0}$, HOU-$C_{18:1}$, HOU-$C_{18:2}$, HOU-$C_{18:3}$ and the control group). Every 10 mice comprised one group. In therapy groups, every mouse was subcutaneously injected with 0.3 ml of HOU-$C_{18:n}$ at a concentration of 200 mg L^{-1} and the control group was injected with the same dose of physiological saline for 7 days. The dose of HOU-$C_{18:n}$ into mice each time was 3 mg kg^{-1}. Two hours after the last injection, 0.5 ml of blood was taken from the eye vein. After 30 min at room temperature, the blood was centrifuged at 3000 rpm for about 10 min, and the serum was separated and used to determine the activity of lysozymes.

3.5.2 Determination of lysozyme activity in serum. *Micrococcus lysodekticus* and standard lysozyme (20,000 U mg^{-1}) were purchased from Nanjing Jiancheng Bioengineering Institute. *Micrococcus lysodekticus* was used as a substrate of lysozyme. Fifty milligrams of powdered *M. lysodekticus* was added to 4 ml 0.05 mol L^{-1} phosphate-buffered saline (PBS, pH 6.2) buffer and ground. The final volume of *M. lysodekticus* was then adjusted to 200 ml with PBS buffer. The final concentration of *M. lysodekticus* was 250 mg L^{-1}. The standard lysozyme was diluted with the same PBS to a final concentration of 100 mg L^{-1}.

One hundred microlitres of separated serum above and 1.5 ml PBS containing 250 mg L^{-1} *M. lysodekticus* were mixed. After incubation for 15 min at 37°C, the reaction was stopped by placing into ice for 3 min and the transmittance of the solution was measured at 530 nm. The rising rate of transmittance indicated the activity of lysozymes. The ratio of lysozyme activity in serum to standard lysozyme activity was indicated by the relative activity.

3.6 Effect of HOU-$C_{18:n}$ on the activity of lysozyme in vitro

HOU-$C_{18:n}$ was added to 50 mmol L^{-1} PBS (pH 6.2) at different concentrations. In sample tube A, 0.5 ml of diluted HOU-$C_{18:n}$, 0.2 ml of standard lysozyme (100 m L^{-1}) and 0.5 ml PBS were mixed and incubated at 37°C. In sample tube B, 0.5 ml of diluted HOU-$C_{18:n}$ and 0.5 ml of *M. lysodekticus* (500 mg L^{-1}) was mixed and incubated at 37°C. After 30 min, samples A and B were mixed and incubated for 15 min at 37°C, and the reaction was stopped by placing into ice for 3 min. One millilitre of HOU-$C_{18:n}$, 0.7 ml PBS and 0.5 ml *M. lysodekticus* (500 mg L^{-1}) were mixed to use as the zero control. One millilitre of HOU-$C_{18:n,}$ 0.5 ml PBS, 0.2 ml standard lysozyme (100 mg L^{-1}) and 0.5 ml *M. lysodekticus* (500 mg L^{-1}) were mixed as the control of the activity of standard lysozyme. The transmittance of the above solution was measured at 530 nm. The rising rate of transmittance indicated the activity of lysozymes. The ratio of lysozyme activity in HOU-$C_{18:n}$ to standard lysozyme activity indicated the relative activity.

3.7 Bacteriostasis of HOU-$C_{18:n}$

HOU-$C_{18:n}$ was diluted into solutions of different concentration with media solution and used to measure the bacteriostasis to *Staphylococcus aureus*, according to the method described in previous reports [2,17].

3.8 Statistical analysis

Experimental data were analysed by DPS statistical software.

Acknowledgement

X.-G.L. was funded by Southwest China Normal University.

References

[1] S. Li, Q.H. Yu, Z.Y. Chu. *Chin. Pharm. Bull.*, **14**, 442 (1998).
[2] X. Ye, X.G. Li, L.J. Yuan, H.M. He. *J. Asian Nat. Prod. Res.* (accepted).
[3] P. Liu, J.M. Tong. *Anim. Sci. Vet. Med.*, **21**, 39 (2004).
[4] Y. Yin, J.S. Peng, W.H. Zhan. *Chin. J. Gastrointest Surg.*, **7**, 233 (2004).
[5] I. Siegel, T.L. Liu, E. Yaghoubzadeh. *J. Natl. Cancer*, **87**, 271 (1987).
[6] M.E. Begin, D. Ells, U.N. Das. *J. Natl. Cancer Inst.*, **77**, 1053 (1986).
[7] R.C. Wander. *J. Nutr.*, **127**, 1198 (1997).
[8] A. Friedman. *Poult Sci.*, **74**, 1463 (1995).
[9] X.G. Li, L.J. Yuan, H.M. He. *China invention patent*, application No. 200410081317.9 (2004).
[10] X.G. Li. *Physical Chemistry of Surfactants and their Application on Agriculture*, pp. 52–70, Southwest China Normal University Public House, China (1997).
[11] T. Mckee, R.M. James. *Biochemistry: An Introduction*, 2nd ed., pp. 240–241, Science Publishing House (2000).
[12] G.Y. Liu, S.M. Chen, R. Wang, Q. Chen. *Chin. Pharm. J.*, **36**, 382 (2001).
[13] W.E. Paul. *Fundamental Immunology*, 4th ed., p. 783, Lippincott-Raven, Philadephia (1999).
[14] X.G. Li. *Acta Biophys. Sin.*, **11**, 13 (1995).
[15] L.T. Xie, J.Q. Huang. *Chin. Tumor Clin.*, **25**, 501 (1998).
[16] L.J. Yuan. Relationship between the antibacterial and immunological activity of houttuyfonate homologues and their surface activities. pp. 47–48, Doctoral Dissertation of Southwest Agricultural University (2004).
[17] H. Li, H.C. Jiang, G.L. Zhou. *Acta Bot. Yunnan*, **24**, 95 (2002).