Transactions of the Materials Research Society of Japan 29[5] 2467-2470 (2004)

Application for the cosmetics material of Hinokitiol-Zinc Stearate Complex and Aomori Hiba neutral oil

Yukako Kitayama, Toshihiro Kanazawa, Yasuhiro Morita*,
Toru Fukui**,and Toshihiro Okabe***
Hiba Development Co.,Ltd, Oma, Aomori 039-4601,Japan
Fax: 0175-32-1011, e-mail: hiba@jomon.ne.jp
*Osaka Organic Chemical Industry, Ltd
**Byotai-Seiri Laboratory
***Industrial Research Institute of Aomori Prefecture

Hinokitiol is contained to Aomori Hiba acid oil. It is known that hinokitiol, which is a tropolone compound, is strongly antibacterial. Its minimum inhibitory concentration (MIC) is 50 to 200 μ g/ml for bacteria and 50 μ g/ml for fungus. However, since it corrodes metal by photolysis, its use has been limited. To solve this problem, hinokitiol can be made to react with an appropriate metal ion to form a metal complex and a salt. Especially, it has been reported that when it is made to react with zinc stearate, an excellent anti-inflammatory property is obtained and hinokitiol's light stability and persistence can be improved while maintaining its antibacterial property. In this research, the simple measuring method of the Hinokitiol content in Aomori Hiba oil was evaluated. Hinokitiol-zinc stearate complex was experimentally produced as a raw material for cosmetics and the following were confirmed: maintenance of the antibacterial property of hinokitiol and improvement in its light stability. The fragrance and antibacterial performance that Aomori Hiba oil has were maintained, by adding Aomori Hiba neutral oil to cosmetics that used Hinokitiol-Zinc Stearate complex.
Key words: hinokitiol-zinc stearate complex, light stability, antimicrobial performance, hinokitiol analysis, Aomori Hiba neutral oil

1. Introduction

The Aomori Hiba, which forms one of the three largest and most beautiful forests in Japan, is a conifer belonging to the Hinoki family Asunaro. Hinokitiol is an acidic, lemon-yellow crystal obtained from Aomori hiba oil, which is distilled from Aomori Hiba sawdust. (The concentration of hinokitiol in Aomori Hiba oil is about 1%.) The application and research of hinokitiol are carried out in the cosmetics, food, medical treatment, agriculture, and other fields because of its excellent antibacterial activity. The structure of hinokitiol is shown in Figure 1 .

On the other hand, because of hinokitiol's photo-instability and strong corrosiveness, its use is limited. There is a method in which a metal complex and also a salt are formed as a solution. Also, it is known that zinc, which also has an antibacterial property, is beneficial to skin because it promotes the old and new metabolism of cells, and backs up collagen generation. However, because the conventional hinokitiol zinc complex is produced from zinc chloride, there is apprehension that the chlorine will damage the user's skin.

In this research, we evaluated the brief measuring method of hinokitiol content by the spectrophotometer. Hinokitiol zinc complex was formed using zinc stearate, which is a metallic soap that is not harmful to skin. The zinc stearate complex samples are compared with conventional technology regarding the light stability, heat stability, and anti-bacterial activities.

2. Experimental method

2.1. Sample material

2.1.1. Natural hinokitiol

The hinokitiol production flowchart is shown in Figure2. Hinokitiol is obtained by distilling hiba oil from sawdust of the Aomori Hiba in steam (1% in the distillation water and 1% in the hiba oil).

Hinokitiol is a 7-membered ring carbon compound and takes the form of acidic lemon-yellow crystals at room temperature. The application and research of hinokitiol are carried out in cosmetics, food, medical treatment, agriculture, and other fields because it has a wide antibacterial spectrum. In this research, natural hinokitiol extracted from hiba oil was used.

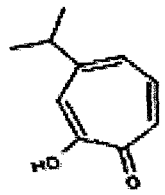

Figure 1 Structure of Hinokitiol

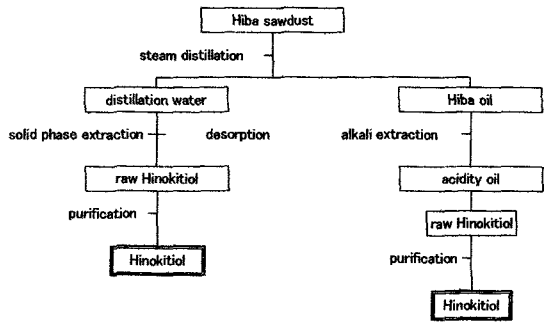

Figure.2 The hinokitiol production flowchart

2.1.2. The hinokitiol zinc stearate ethanol pharmaceutical.

The pharmaceutical hinokitiol zinc-stearate preparation shown in Table1 was prepared. First, glycerin (3%) was added to ethanol (93.2%) and the mixture was agitated. Then, hinokitiol (2%) and then zinc stearate (1.8%) were dissolved in the mixture. The procedure was performed at about 40℃.

Table.1 The hinokitiol zinc stearate ethanol pharmaceutical

	wt%
Ethanol	93.2
Glycerin	3.0
Hinokitiol	2.0
Zinc Stearate	1.8

2.1.3 Aomori hiba neutral oil

Aomori hiba is divided to the acid oil (8%) including Hinokitiol and neutral oil (92%) (Figure.3) when it separates with alkali aqueous solution. The sesquiterpene is main and the most is Thujopsene.

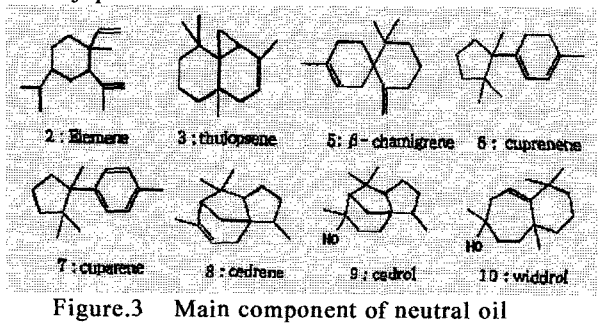

Figure.3 Main component of neutral oil

2.2 Hinokitiol analysis

The Hinokitiol content by the HPLC of 5 kinds of unknown hiba oil is measured.

5 kinds of unknown hiba oil are diluted to 1000 times with ethanol. The absorbance of 330nm is measured by the spectrophotometer.

I obtain the calibration curve from the result of high performance liquid chromatography and the result of the spectrophotometer.

2.3 Light-stability test

Hinokitiol and the hinokitiol zinc complex were diluted in ethanol to 16 ppm. The hinokitiol concentration in the hinokitiol zinc-stearate preparation was adjusted to 16 ppm. Then, 50 ml quantities of the mixture were put into laboratory dishes, which were then covered with glass lids. Then, the dishes were exposed to 5,000 Lux of light from a fluorescent lamp (Mitsubishi BB Giraffe inverter). Next, 3 ml samples were removed from the dishes at 0, 1, 3, and 5 hours after starting irradiation and the absorbance at the 1st peak (240 nm) and 2nd peak (330 nm) of hinokitiol's light absorption spectrum were measured with a spectrophotometer (Shimazu Uvmini-1240 ultraviolet visibility spectrophotometer).

2.4 Heat-instability test

Measurements were made using Seiko Instruments' EXSTAR 6000 PC station thermal analysis system.

Sample weight:2.90mg
Stomosphere:N2
Flow rate:100ml/min
Heating rate:5℃/1min
Sampling:solid

2.5 Antibacterial activity test

The antibacterial property of hinokitiol, the hinokitiol zinc complex, and hinokitiol zinc-stearate preparations were investigated.For this test,four types of hinokitiol zinc-stearate preparations shown in Table 2 were prepared.

Table.2 Preparation methods for hinokitiol zinc-stearate complex

(wt%)	①	②	③	④
Ethanol	93.2	96.2	91.5	93.2
Glycerin	3.0	-	3.0	-
Hinokitiol	2.0	2.0	2.5	2.0
Zinc stearate	1.8	1.8	3.0	1.8
Propylene glycol	-	-	-	3.0

The minimum inhibitory concentrations (MIC) for standard strains of Staphylococcus aureus (Staphylococcus aureus ATCC 25923) and coli bacteria (Escherichia coli ATCC 25922) were determined. The tests were done using cultures that were grown overnight in Mueller Hinton culture medium at 35℃. The

culture medium was diluted with sterilized physiological saline to obtain solutions of about 10^5 CFU/ml as bacteria liquid for the test. Dimethyl sulfoxide (DMSO) was used to obtain 10 mg/ml solutions of the hinokitiol and hinokitiol complex preparations, and sterilized water was used to adjust the concentration of the solutions by diluting them twice. Then, 0.3 ml of the solution was poured into each hole on sterilized 24-hole tissue culture plates and the solution in the holes was diluted with 0.3 ml of a sterilized 1/2 solution of the culture medium for dry disk (Nissui Pharmaceutical) at about 50℃. The surfaces of these discoid culture media were dried for 30 minutes and then inoculated with 10 μL of the bacteria liquid. The bacteria were incubated on the culture media at 35℃ overnight, and then the minimum inhibitory concentrations (MIC) were measured. Discoid culture media using distilled water instead of the preparations were used as contrasts.

3. Results and discussion

3.1 Hinokitiol analysis

Hinokitiol content by HPLC and the absorbance by the spectrophotometer proportioned (Figure.4). By using this calibration curve we can obtain hinokitiol content briefly by the spectrophotometer.

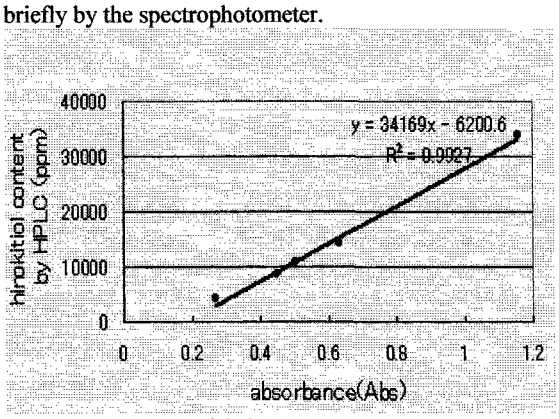

Figure.4 Hinokitiol content analysis

3.2 Light-stability test

The results are shown in Figure5. Hinokitiol zinc stearate complex (HT-Zn) was stabler in light than Hinokitiol (HT). Hinokitiol-zinc stearate complex (HT-SZn) was stabler in light than hinokitiol zinc complex. HT-SZn ① is stabler than HT-SZn ②. It is conceivable ①'s stability according to the dispersion effect of glycerin.

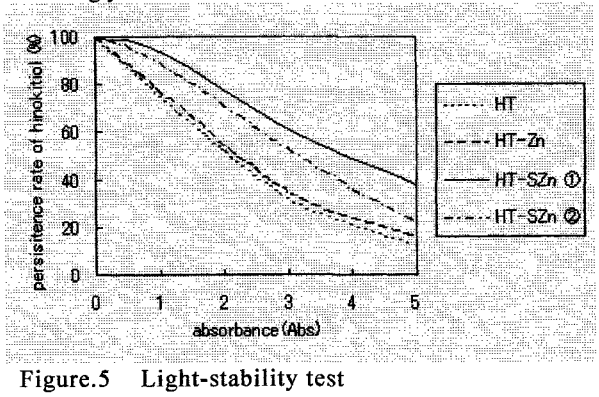

Figure.5 Light-stability test

3.3 Heat-instability test

The thermal analysis of hinokitiol is shown in Figure.6. There were endothermic peaks near 50℃ and 170℃. This result agrees with the fact that hinokitiol is sublimated near 50℃. The thermal analysis of the hinokitiol zinc-stearate complex is shown in Figure.7. The endothermic peaks of hinokitiol zinc-stearate complex are near 110℃, 370℃. The endothermic peaks deviated to high temperature. These results show that the hinokitiol zinc-stearate complex is more thermally stable than the pure hinokitiol.

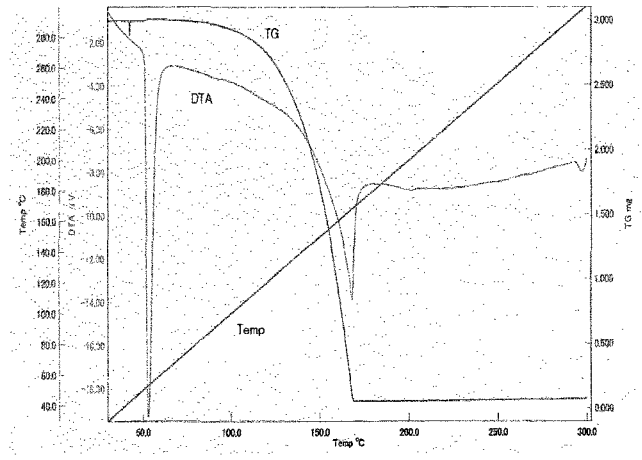

Figure.6 Thermal analysis of hinokitiol

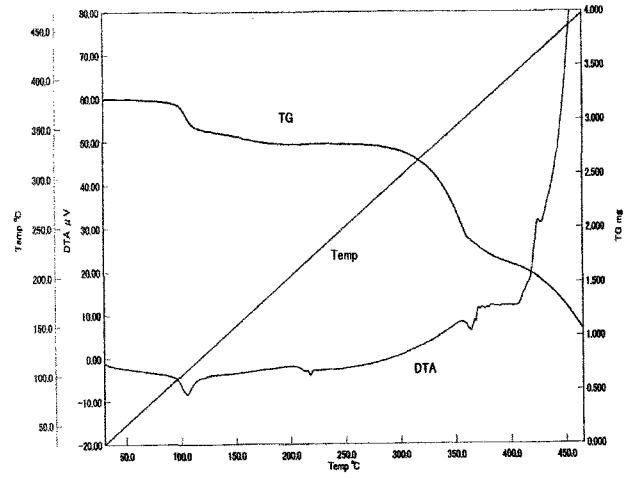

Figure.7 Thermal analysis of hinokitiol zinc-stearate complex

3.4 Antibacterial activity test

The results of the antimicrobial test are shown in Table 3. Compared to pure hinokitiol, the hinokitiol zinc-stearate complex has a stronger antibacterial activity against S. aureus ATCC25923 and E. coli ATCC25922. It is reasonable to assume that the stronger activity is due to the presence of zinc combined

to hinokitiol. Also, it is reasonable to conclude that the hinokitiol zinc stearate complex is a useful material because it maintains the antibacterial power of a conventional hinokitiol zinc complex.

Table.3 Minimum inhibitory concentrations (MIC)

	S. aureus ATCC25923	E. coli ATCC25922
HT	100	100
HT-Zn	31.3	15.6
HT-SZn ①	31.3	15.6
HT-SZn ②	31.3	15.6
HT-SZn ③	31.3	15.6
HT-SZn ④	31.3	31.3

(μ g/ml)

4.Summary

- We were able to obtain the calibration curve of the Hinokitiol content in hiba oil by HPLC and spectrophotometer. The Hinokitiol content in hiba oil was able to be measured briefly by the spectrophotometer.
- Hinokitiol zinc-stearate complex was stabler in light than hinokitiol zinc complex.
- Hinokitiol zinc-stearate complex is more thermally stable than hinokitiol.
- The hinokitiol zinc-stearate complex has a stronger antibacterial activity against S. aureus ATCC25923 and E. coli ATCC25922. It is a stronger anti bacteria activity than hinokitiol.
- By using Aomori hiba neutral oil we were able to add the fragrance that has a mind stable effect. The application of neutral oil is connected to the advanced utilization of hiba oil.

References

[1] S. Matsumoto, T. Fukui, S. Terasaki, S. Yoshinaka, A. Hasegawa, T. Okabe, K. Saito, K. Iinuma.: Antibacterial activities of metal chelates of Hinokitiol. J. Antibact. Antifung. Agents Vol.22, No.5, 265 to 269 (1994)

[2] T. Okabe, K. Saito, Yotomo.: Physiochemical property and antibacterial action of the wood constituent hinokitiol. Food Chemical, 45 to 52 (1988-2)

[3] T. Okabe, K. Saito.: The pharmacological effect of wood extractions. Wood Conservation, Vol.19-2 (1993)

(Received October 11, 2003; Accepted December 11, 2003)