Journal of the Korean Chemical Society
2024, *Vol. 68, No. 4*
Printed in the Republic of Korea
https://doi.org/10.5012/jkcs.2024.68.4.199

Antimicrobial Terpenoids from Seed of *Chamaecyparis obtusa* (Siebold & Zucc.) Endl.

Bo Shi Liu, Jung Eun Kim, and Nam Ho Lee*
Department of Chemistry and Cosmetics, Jeju National University, Jeju 63243, Korea.
**E-mail: namho@jejunu.ac.kr*
(Received June 24, 2024; Accepted July 13, 2024)

ABSTRACT. *Chamaecyparis obtusa* (Siebold & Zucc.) Endl. is an evergreen tree of the family Cupressaceae well known for its unique scents. The seed extract of this cypress tree was phytochemically investigated to isolate a novel abietane-diterpene compound (**1**) along with fifteen known terpenoids (**2-16**). All of the isolated compounds were subjected to the screening of antimicrobial activities against *Cutibacterium acnes* and *Staphylococcus epidermidis* including erythromycin resistant strains. Among the isolates, 1α-hydroxy-hinokione (**1**), hinokione (**3**), 1,2-dehydrohinokione (**4**) and ferruginol (**9**) showed significant antibacterial activities against both acne-causing strains. This study demonstrated that abietane-type diterpenoids are the main antibacterial components in *C. obtusa* seed extract, and some isolated compounds can be further developed as potential acne-treatment agents.

Key words: *Chamaecyparis obtusa*, Antimicrobial, Abietane-diterpenoid, *Cutibacterium acnes*, *Staphylococcus epidermidis*

INTRODUCTION

Acne is a chronic inflammatory disease occurring in the sebaceous glands of the skin.[1] The Gram-positive bacteria such as *Cutibacterium acnes* and *Staphylococcus epidermidis* are directly involved in the process of causing acne. In fact, *C. acnes* secretes lipolytic enzymes to break down the triglycerides of sebum to form free fatty acids, which stimulate the skin to cause inflammation in the cells around the pores.[2] Meanwhile, *S. epidermidis* normally distributed in the skin is not a major cause of inflammation, but is known to play a role in exacerbating acne by further expanding the inflammatory areas.[3]

Among the ways to treat acne, antibiotics such as erythromycin and clindamycin are locally applied to the skin to suppress the proliferation of strains.[4] However, this type of treatment requires a long period of time and then causes antibiotic-resistant bacteria along with several side effects.[5] In practice, the occurrence of resistant bacteria is recognized as a limitation in antibiotic treatment in acne.[6]

Natural products attract attention as a way to develop treatment with fewer side effects in the field of skin diseases.[7] Naturally derived microbial compounds are especially attractive in solving problems related to resistant bacteria.[8]

Chamaecyparis obtusa (Siebold & Zucc.) Endl. is an evergreen tree of the family Cupressaceae growing up to 40 meters in height. This cypress tree is native to Japan, but has been planted long ago to build a forest in southern area of Korea including Jeju Island.[9] These trees are widely used as indoor decoration wood and household goods due to their own unique fragrance. As the main cause of these scents, terpene compounds have been identified in the leaves and branches as well as in seeds. In addition to research on terpene ingredients, studies on the antibacterial effect of *C. obtusa* have also been revealed.[10-14] However, there are no reports on the verification of the antibacterial effect on the strain *C. acnes* related to acne treatment.

In this study, we tried to find natural products possessing activities against *C. acnes* and *S. epidermidis* including erythromycin resistant strains from cypress tree seeds. As a result of phytochemical studies, identified were one new diterpene compound (**1**) as well as 15 known terpenoids (**2-16**), and some of which exhibit potent antimicrobial activities.

EXPERIMENTAL

General Experimental Procedures

All solvents of analytical grade were used without further purification. Optical rotation was measured with Model 343 polarimeter (λ 589 nm (D), 20 ℃, PerkinElmer Inc., USA). HR-ESI-MS data was performed on the SYNAPT G2 mass spectrometer (Waters Co., USA).1D and 2D NMR spectra were performed on JNM-EXC 400 (FT-NMR system, 400 MHz, JEOL Co., Japan) using $CDCl_3$, CD_3OD,

acetone-d_6 and pyridine-d_5 as a solvent for measurement. The chemical shift values are reported in ppm relative to the solvent used. Sephadex^{TM} LH-20 (GE healthcare Co., Sweden) and silica gel 60 (Merck Co., Germany) were used for column chromatography, and precoated silica gel 60 F-254 plates (Merck Co., Germany) were used for thin-layer chromatography (TLC). The spots on TLC were detected by spraying with 3% $KMnO_4$ aqueous solution and anisaldehyde solution with 5% H_2SO_4 and then heating on a hot plate. Bacto^{TM} tryptic soy agar (TSA), and Bacto^{TM} tryptic soy broth (TSB) were purchased from Difco (USA). Gifu Anaerobic Medium (GAM) broth was purchased from MB cell (Korea).

Plant Material

The seeds of *C. obtusa* were collected in Gwangpyeong-ri, Seogwipo city of Korea, in December 2019. Voucher specimen (No. 497) was deposited at Natural Product Chemistry Laboratory, Department of Chemistry and Cosmetics, Jeju National University.

Extraction and Isolation

The seeds of *C. obtusa* (0.5 kg) were extracted two times with 50% aqueous ethanol (4.0 L) under stirring for 24 h at room temperature. The extracted solution was filtered, and the filtrate was concentrated under reduced pressure and freeze dried to afford a tan powder (25.5 g). A portion of the extract (20.7 g) was suspended in water (2.0 L) and fractionated into *n*-hexane (Hex, 6.3 g), ethyl acetate (EtOAc, 6.9 g), *n*-butanol (BuOH, 2.8 g) and water (H_2O, 4.0 g) portions.

The *n*-Hex soluble fraction (4.8 g) was subjected to vacuum liquid chromatography (VLC) over silica gel using step gradient solvents (*n*-Hex-EtOAc-methanol) to give 21 subfractions (VH1 to VH21). The fraction VH14 (222.2 mg) was further purified using Sephadex LH-20 column chromatography (CC) with chloroform-methanol (30:1) eluents to afford compound **1** (49.7 mg). The fraction VH6 (645.4 mg) was chromatographed by silica gel with chloroform-methanol (80:1) eluents to afford compounds **12** (32.2 mg), **15** (29.6 mg) and **14** (17.9 mg). A Fr. VH9 (820.3 mg) was further purified using Sephadex LH-20 CC with chloroform-methanol (60:1) eluents to yield compounds **3** (29.7 mg), **4** (45.2 mg) and **16** (84.8 mg). A Fr. VH10~11 (560.2 mg) was also purified using Sephadex LH-20 CC with chloroform-methanol (50:1) eluents to give compounds **2** (58.3 mg) and **7** (49.8 mg). The compound **8** (50.4 mg) was obtained from fraction VH7 (240.8 mg) by recrystallization with methanol. A fraction VH12~13 (777.0 mg) was further purified using Sephadex LH-20 CC with chloroform-methanol (40:1) eluents to afford compound **6** (45.8 mg). The compounds **9** (68.7 mg) and **10** (110.8 mg) were obtained from the fraction VH4 (226.5 mg) by silica gel CC using chloroform eluent.

The EtOAc soluble fraction (5.0 g) was also subjected to VLC over silica gel using step gradient solvents (*n*-hexane-EtOAc-methanol) to give 32 subfractions (VE1 to VE32). A fraction VE4~5 (310.5 mg) was further purified using silica gel CC with *n*-Hex-EtOAc (10:1) eluents to give compound **13** (9.8 mg). Silica gel CC of the fraction VE6 (230.4 mg) with chloroform-methanol (60:1) led to isolation of the compound **11** (7.1 mg). A fraction EV18~19 (350.3 mg) was purified using Sephadex LH-20 CC with chloroform-methanol (15:1) eluents to afford compound **5** (16.9 mg).

Antimicrobial Activities against *C. acnes* and *S. epidermidis*

The strains of *C. acnes* (CCARM 0081, 9009, 9010, 9089) and *S. epidermidis* (CCARM 3709, 3710, 3711) were purchased from the Culture Collection of Antimicrobial Resistant Microbes (CCARM, Korea). *C. acnes* were cultured in GAM in anaerobic condition using Becton Dickinson Gas Pak^{TM} EZ gas generating container system. Subculture of *C. acnes* was conducted every 48 h in a 37 ℃ incubator. *S. epidermidis* were cultured in TSB and TSA. Subculture of *S. epidermidis* was conducted every 24 h under aerobic conditions in a 37 ℃ incubator.

The experimental protocol accompanied a previous report with modifications.[15] Ethanol extract, *n*-Hex, EtOAc, *n*-BuOH and H_2O fractions as well as isolated compounds **1**-**16** of *C. obtuse* were evaluated against *C. acnes* by determining the MIC values through the broth-dilution method. A freshly grown culture was diluted with GAM and prepared bacteria (2×10^5 CFU/mL). Erythromycin was tried as a positive control. Samples were diluted in sterile broth and subsequently mixed with broth incubated with *C. acnes*. Dilutions were proceeded by 2-fold until to the desired final concentration. The test concentrations of ethanol extract, *n*-Hex, EtOAc, *n*-BuOH, and H_2O fractions were the range of 4,000-3.91 μg/mL. The test concentrations of isolated compounds were the range of 4,000–3.91 μM. Test samples were incubated under anaerobic conditions at 37 ℃ for 48 h until visible growth of the test microorganisms was observed in the control. The O.D. value was measured using a microplate reader at 600 nm. Also, *S. epidermidis* (2×10^5 CFU/mL) was prepared, and the test concentrations of 50% EtOH extract, *n*-Hex, EtOAc, *n*-BuOH, and H_2O fractions were 10,000-9.76 μg/mL. Test samples were

incubated at 37 ℃ for 24 h until visible growth of the test microorganisms was observed in the control.

RESULTS AND DISCUSSION

Isolation and Structure Elucidation of Compounds

The *n*-Hex and EtOAc fractions of the 50% aqueous ethanol extract from *C. obtusa* seed were subjected to repeated column chromatography over silica gel and Sephadex LH-20 eluting with various solvent systems to afford one novel abietane-diterpene (**1**) along with fifteen known terpenoids (**2-16**, *Fig.* 2). The NMR spectra for the isolates (**1-16**) can be found in supplementary materials.

Compound **1** was obtained as a white needle crystal. Its molecular formula was determined as $C_{20}H_{28}O_3$ according to the $[M+Na]^+$ peak at m/z 339.1937 (Calcd. 339.1936) in the positive HR-ESI-MS, indicating 7 degrees of unsaturation. The specific optical rotation was $[\alpha]^{20}_D = +45.2$ (*c* 0.2, $CHCl_3$).

Inspection of ^{13}C-NMR and DEPT-135° spectra revealed the presence of total 20 carbons including 3 methylene carbons (δ_C 42.1, 30.5, 20.2), 7 quaternary carbons (δ_C 215.8, 151.8, 140.5, 133.4, 129.9, 47.3, 43.6) and 10 methyl and methine carbons (*Table* 1). Among them, the peak of δ_C

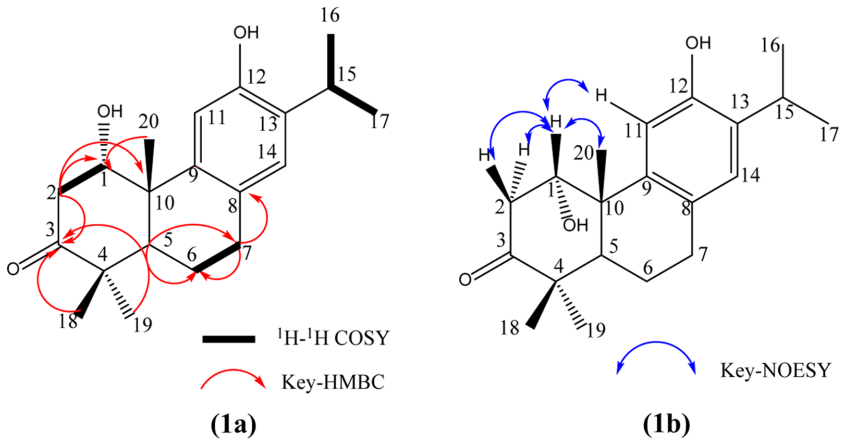

***Figure* 1.** ^{1}H-^{1}H COSY and key HMBC correlation (a) and key NOESY correlation (b) in the compound **1**.

***Table* 1.** 1D and HMBC (H → C) data for compound **1** in $CDCl_3$ (400 MHz)

No	δ_H (int, mult, *J* Hz)	δ_C	HMBC (J^2)	HMBC (J^3)
1	4.50 (1H, dd, 5.0, 3.2)	74.3		
2	3.01 (1H, dd, 16.5, 5.0), 2.65 (1H, dd, 16.5, 3.2)	42.1	74.3 (C1), 215.8 (C3)	43.6 (C10)
3		215.8		
4		47.3		
5	2.34 (1H, dd, 11.9, 2.8)	43.5	47.3 (4), 20.2 (C6), 43.6 (C10)	30.5 (C7), 21.4 (C18), 26.7 (C19), 22.8 (C20)
6	1.70~1.83 (2H, m)	20.2		43.6 (C10)
7	2.77 (1H, ddd, 16.6, 11.9, 5.0), 2.85 (1H, ddd, 16.6, 5.0, 2.8)	30.5	20.2 (C6), 129.9 (C8)	
8		129.9		
9		140.5		
10		43.6		
11	6.64 (1H, s)	110.8	151.8 (C12)	129.9 (C8), 43.6 (C10), 133.4 (C13)
12		151.8		
13		133.4		
14	6.89 (1H, s)	128.2		30.5 (C7), 140.5 (C9),151.8 (C12), 27.0 (C15)
15	3.10 (1H, sept, 6.9)	27.0	133.4 (C13), 24.2 (C17)	151.8 (C12), 128.2 (C14)
16	1.21 (3H, d, 6.9)	22.7	27.0 (C15)	133.4 (C13), 24.2 (C17)
17	1.23 (3H, d, 6.9)	24.2	27.0 (C15)	133.4 (C13), 22.7 (C16)
18	1.11 (3H, s)	26.7	47.3 (C4)	43.5 (C5), 215.8 (C3), 21.4 (C19)
19	1.16 (3H, s)	21.4	47.3 (C4)	43.5 (C5), 215.8 (C3), 26.7 (C18)
20	1.24 (3H, s)	22.8	43.6 (C10)	74.3 (C1), 140.5 (C9)

215.8 is expected to be from a carbonyl group, and the remaining 6 sp^2 carbons indicate the presence of an aromatic ring with a phenolic hydroxyl group presumed based on the deshielded signal (δ_C 151.8). The signal at δ_C 74.3 is a characteristic of the aliphatic carbon directly attached to the oxygen atom. ^{1}H-NMR spectral analysis of compound **1** shows that the signals at δ_H 6.89 (1H, s) and 6.64 (1H, s) are from aromatic protons and the signals at δ_H 1.11-4.05 are from aliphatic carbon-linked protons, where the signal at δ_H 4.50 (1H, dd, J = 5.0, 3.2 Hz) predicts the presence of oxygen-bound aliphatic carbon. The deshielded septet splitting of the peak δ_H 3.10 (1H, J = 6.9 Hz) is a characteristic of isopropyl proton attached to the sp^2 carbon. Five methyl proton signals were identified by the integrated value at δ_H 1.22 (3H, d, J = 6.9 Hz), 1.21 (3H, d, J = 6.9 Hz), 1.24 (3H, s), 1.16 (3H, s) and 1.11 (3H, s). Judging by the information of 20 carbon atoms, the compound **1** was presumed to have the abietane-type diterpene framework. The suggested structure was further conformed by COSY and HMBC spectrum (*Fig.* 1a). The HMBC connections of the carbon at carbonyl group (δ_C 215.8) were established with the aliphatic carbons at δ_C 42.1, 26.7 and 21.4. Also, oxygen-bearing δ_C 74.3 was connected to the aliphatic carbons δ_C 42.1 and 22.8, which confirmed that hydroxy group is in the C-1 position and the carbonyl is in the C-3 position in the diterpene skeleton. The relative configuration of hydroxy group at C-1 was established on the basis of the NOESY spectrum (*Fig.* 1b). The proton at the C-1 shows NOESY cross-peaks with the aromatic ring proton at the C-11, the methyl proton at C-20 position and the aliphatic proton of C-2 position, thus confirming the configuration of hydroxy group in axial (α) position. Based on these results, compound **1** was named 1α-hydroxy-hinokione, which was first isolated in nature.

Further phytochemical studies of the fractions led to the isolation of fifteen known terpene compounds (*Fig.* 2); 12-methoxy-8,11,13-abietatriene-7β,11-diol-3-one (**2**),[12] hinokione (**3**),[16] 1,2-dehydrohinokione (**4**),[17] 1α-hydroxy-hinokiol (**5**),[18] hinokiol (**6**),[19] isohinokiol (**7**),[20] sugiol (**8**),[21] ferruginol (**9**),[22] cryptojaponol (**10**),[23] 7α,11-dihydroxy-12-methoxy-8,11,13-abietatriene(**11**),[24] 7β-hydroxydeoxocryptojaponol (**12**),[25] 6,7-dehydrodeoxocryptojaponol (**13**),[26] *trans*-communic acid (**14**),[27] α-eudesmol (**15**)[28] and hinokiic acid (**16**).[29] Besides abietane-type diterpenes (**1**-**13**), labdane-type diterpene **14** and sesquiterpens (**15**, **16**) were identified. All of these compounds were elucidated based

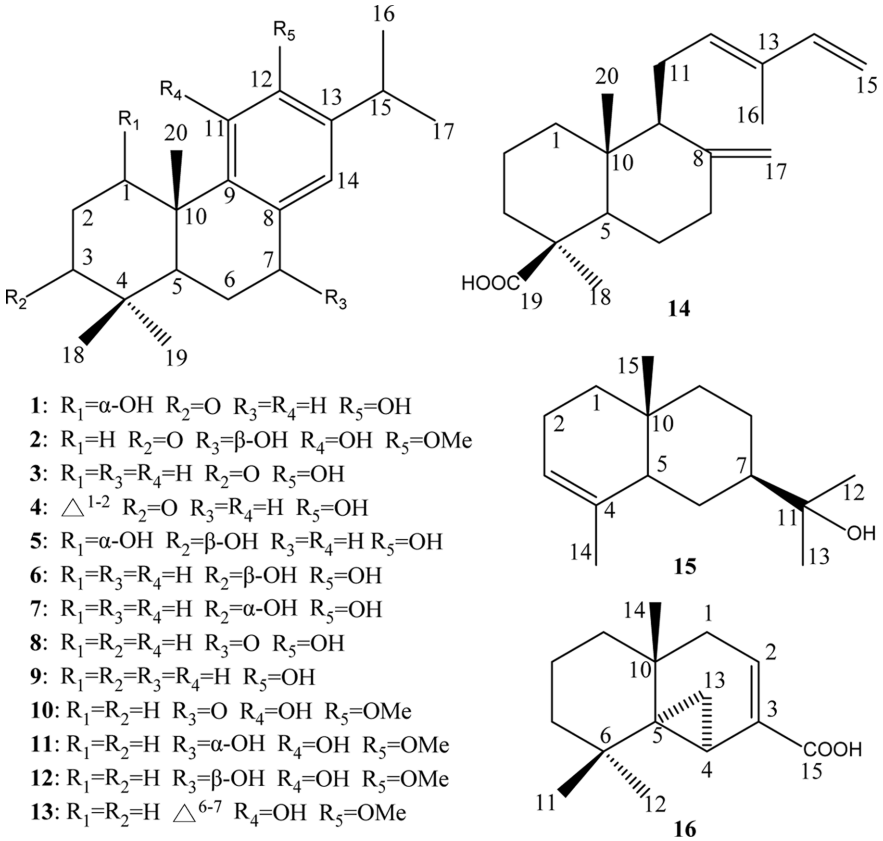

***Figure* 2.** Isolated compounds **1**-**16** from the seeds of *C. obtuse*.

on the NMR studies as well as comparison of the spectral data to the literature values.

Antimicrobial Activities of Solvent Fractions and Isolated Compounds (1-16)

As the bacterial strains on the skin are closely involved in the occurrence of acne, examination of the antimicrobial activities for the plant extracts was conducted as the first step in order to develop natural acne treatment. In this experiment, the extract and solvent fractions (*n*-hexane, ethyl acetate, *n*-butanol and water fr.) of *C. obtusa* seed were subjected to screening of antimicrobial activities against *C. acnes* (CCARM 0081, 9009, 9010, 9089) and *S. epidermidis* (CCARM 3709, 3710, 3711) by determining the inhibition ratio using the broth dilution method. Among the strains, CCARM 9009, 9010 and 3710 possess a resistance to erythromycin, an antibiotics used clinically to treat acne. The results showed that the seed extracts and their non-polar fractions (*n*-Hex and EtOAc fr.) have significant antimicrobial activities against *C. acnes* (MIC 31.2 - 62.5 μg/mL) and *S. epidermidis* (MIC 156.25 - 2500 μg/mL) including resistant strains (the data were provided in supplementary material). On the other hand, the polar fractions (*n*-BuOH and H_2O fr.) showed very low activities with MIC values over 1000 μg/mL.

As previously mentioned, separation and purification was carried out using the active fractions (*n*-Hex and EtOAc fr.) leading to identify sixteen terpenoids (**1-16**). In the subsequent studies, the antimicrobial activities for each isolates (**1-16**) were screened against *C. acnes* and *S. epidermidis* (*Table* 2) respectively. In an activity experiment with *C. acnes*, all of the isolated compounds except hinokiol (**6**) showed good to moderate antibacterial activities. As a whole, the diterpenes (**1-14**) exhibited better antibacterial activities compared to the sesquiterpenes (**15**, **16**), indicating that the abietane-type diterpenes are the main antimicrobial components of *C. obtusa* seeds extract. The ferruginol (**9**) showed the most potent activities against resistant strains with MIC value of 7.81 μM. Diterpene compounds 1,2-dehydrohinokione (**4**), 7β-hydroxydeoxocryptojaponol (**12**), and *trans*-communic acid (**14**) also exhibited excellent activities with MIC of 15.6 - 31.2 μM. On the other hand, in an experiment with *S. epidermis*, the terpenoids showed relatively very lower antimicrobial activities compared to the test with *C. acnes*. The best activity here was also observed in the ferruginol (**9**) with MIC of 15.6 μM. Only three compounds such as 1α-hydroxy-hinokione (**1**), hinokione (**3**), and 1,2-dehydrohinokione (**4**) other than **9** were observed as active substances with minimum inhibitory concentration (MIC) lower than 1000 μM. Analyzing the relationship between chemical structure and activity here revealed that the phenol at the C-12 position is the key fac-

***Table* 2.** MIC values of isolated compounds **1-16** from *C. obtusa* seed on *C. acnes* and *S. epidermidis*

	MIC (μM)						
	C. acnes				*S. epidermidis*		
	CCARM 0081	CCARM 9009[1]	CCARM 9010[1]	CCARM 9089	CCARM 3709	CCARM 3710[1]	CCARM 3711
1	15.62	62.5	62.5	31.25	250	250	250
2	250	250	250	250	>4000	4000	>4000
3	15.62	62.5	62.5	62.5	125	125	250
4	15.62	15.62	31.25	15.62	62.5	62.5	62.5
5	125	250	250	250	1000	1000	1000
6	>4000	>4000	>4000	>4000	>4000	>4000	>4000
7	250	500	1000	500	4000	>4000	>4000
8	1000	1000	1000	1000	>4000	>4000	>4000
9	7.81	7.81	7.81	15.62	15.62	15.62	31.25
10	125	1000	500	500	4000	4000	>4000
11	31.25	62.5	125	125	>4000	>4000	>4000
12	15.62	31.25	31.25	31.25	1000	1000	1000
13	31.25	31.25	500	250	4000	4000	4000
14	31.25	31.25	31.25	31.25	500	1000	2000
15	62.5	125	125	125	1000	1000	1000
16	250	250	250	250	2000	1000	2000
Erythromycin[2]	<3.90	>4000	>4000	<3.90	<3.90	>10000	<3.90

[1]Erythromycin-resistant strains
[2]Positive control

tor for better activity against *S. epidermidis*, as observed in the previous report.[30] As shown results in compounds **1**, **3** and **4**, substitution of carbonyl instead of hydroxyl at the C-3 seems to be also important in exhibiting better activities.

CONCLUSION

Chamaecyparis obtusa is an evergreen tree of the family Cupressaceae widely distributed on Jeju Island. In this study, the extracts and solvent fractions from *C. obtusa* seeds were investigated on antibacterial activities against *C. acnes* and *S. epidermidis* including resistant strains. As the results of the experiment, good to moderate activities were observed in *n*-Hex and EtOAc fractions. Phytochemical studies were conducted for the two fractions leading to identification of an abietane diterpene 1α-hydroxy-hinokione (**1**) as a new compound. In addition, fifteen known compounds were isolated; 12-methoxy-8,11,13-abietatriene-7β,11-diol-3-one (**2**) hinokione (**3**), 1,2-dehydrohinokione (**4**), 1α-hydroxy-hinokiol (**5**), hinokiol (**6**), isohinokiol (**7**), sugiol (**8**), ferruginol (**9**), cryptojaponol (**10**), 7α,11-dihydroxy-12-methoxy-8,11,13-abietatriene (**11**), 7β-hydroxydeoxocryptojaponol (**12**), 6,7-dehydrodeoxocryptojaponol (**13**), *trans*-communic acid (**14**), α-eudesmol (**15**), hinokiic acid (**16**). Among the isolated compounds (**1-16**), a phenolic diterpene ferruginol (**9**) was found to have uniquely potent antibacterial activities against both *C. acnes* and *S. epidermidis* at the same time. Its activities were excellent regardless of bacterial resistance strains. Subsequently, as shown in *Table* 2, diterpenes **1**, **3** and **4** exhibited good activities in both strains *C. acnes* and *S. epidermidis*. Compounds **12** and **14** showed strong activities in *C. acnes*, whereas they showed little activities in *S. epidermidis*. In conclusion, *C. obtusa* seed extracts containing terpenoids 1α-hydroxy-hinokione (**1**), hinokione (**3**), 1,2-dehydrohinoki-one (**4**) and ferruginol (**9**) can be applied as anti-acne ingredients in pharmaceutical and/or cosmetic preparations.

Acknowledgments. This research was supported by the 2023 scientific promotion program funded by Jeju National University.

Supporting Information. Additional supporting information (NMR data for the compounds **1-16**) is available in the online version of this article.

REFERENCES

1. Sousa, I. C. V. D. D. *Expert Opin. Pharmacother.* **2019**, *20*, 1009.
2. Beylot, C.; Auffret, N.; Poli, F.; Claudel, J. P.; Leccia, M. T.; Giudice, P. D.; Dreno, B. *J. Eur. Acad. Dermatol. Venereol.* **2014**, *28*, 271.
3. Dong, Y.; Speer, C. P.; Glaser, K. *Virulence* **2018**, *9*, 621.
4. Nguyen, T. A.; Eichenfield, L. F. *Clin. Cosmet. Investig. Dermatol.* **2015**, *8*, 549.
5. Leyden, J. J.; Rosso, J. Q. D.; Webster, G. F. *Cutis* **2007**, *79*, 9.
6. Mancuso, G.; Midiri, A.; Gerace, E.; Biondo, C. *Pathogens* **2021**, *10*, 1310.
7. Fernandes, A.; Rodrigues, P. M.; Pintado, M.; Tavaria, F. K. *Phytomedicine* **2023**, *115*, 154824.
8. Rossiter, S. E.; Fletcher, M. H.; Wuest, W. M. *Chem. Rev.* **2017**, *117*, 12415.
9. Kim, T. L.; Lee, K.; Cho, W.; Park, D.; Lee, I. H.; Lim, H. *Plan. Bre. Biote.* **2021**, *9*, 112.
10. Park, M. J.; Lee, S. M.; Gwak, K. S.; Jeung, E. B.; Chang, J. W.; Choi, I. G. *J. Korean Wood Sci. Technol.* **2005**, *33*, 72.
11. Morikawa, T.; Ashitani, T.; Sekine, N.; Kusumoto, N.; Takahashi, K. *J. Wood Sci.* **2012**, *58*, 544.
12. Ozaki, N.; Hasegawa, S.; Hirose, Y. *Phytochemistry* **1983**, *22*, 1771.
13. Yang, J. K.; Choi, M. S.; Seo, W. T.; Rinker, D. L.; Han, S. W.; Cheong, G. W. *Fitoterapia* **2007**, *78*, 149.
14. Kwon, Y. J.; Seo, E. B.; Kim, S. K.; Noh, K. H.; Lee, H.; Joung, Y. W.; Shin, H. M.; Jang, Y. A.; Kim, Y. M.; Lee, J. T.; Ye, S. K. *J. Ethnopharmacol.* **2022**, *282*, 114493.
15. Jones, R.; Barry, A. L.; Gavan, T. L.; Washington, J. *Manu. Clini. Microbi.* **1985**, 972.
16. Asano, N.; Yamamoto, H. *Bull. Col. Edu. Ibaraki Univ. (Nat. Sci.)* **1994**, *43*, 61.
17. Yamamoto, H.; Yamaguchi, S.; Murakami, Y.; Hosoda, H. *Bull. Col. Edu. Ibaraki Univ. (Nat. Sci.)* **2008**, *57*, 77.
18. Mossa, J. S.; Muhammad, I.; El-Feraly, F. S.; Hufford, C. D. *Phytochemistry* **1992**, *31*, 2789.
19. Li, B. L.; Tian, G. H.; Zhang, Z. G.; Liang, B.; Wang, W. *Chem. Nat. Compd.* **2007**, *43*, 274.
20. Wang, S. Y.; Wu, J. H.; Shyur, L. F.; Kuo, Y. H.; Chang, S. T. *Holzforschung* **2002**, *56*, 487.
21. Bajpai, V. K.; Kang, S. C. *J. Food Safe.* **2011**, *31*, 276.
22. Lee, S. Y.; Choi, D. Y.; Woo, E. R. *Arch. Pharm. Res.* **2005**, *28*, 909.
23. Rodríguez, B. *Magn. Reson. Chem.* **2003**, *41*, 741.
24. Dellar, J. E.; Cole, M. D.; Waterman, P. G. *Phytochemistry* **1996**, *41*, 735.
25. Kuo, Y. H.; Lin, N. H.; Lin, Y. T. *J. Chin. Chem. Soci.* **1980**, *27*, 19.
26. Kuo, Y. H.; Wu, T. R.; Cheng, M. C.; Wang, Y. *Chem. Pharm. Bull.* **1990**, *38*, 3195.
27. Fukushima, J. I.; Yatagai, M.; Ohira, T. *J. Wood Sci.* **2002**, *48*, 326.
28. Tebbaa, M.; Hakmaoui, A. E.; Benharref, A.; Akssira, M. *Tetrahedron Lett.* **2011**, *52*, 3769.
29. Swartley, D. B. Master of Science, Oregon State University, Corvallis, USA, **2002**.
30. Yang, Z.; Kitano, Y.; Chiba, K.; Shibata, N.; Kurokawa, H.; Doi, Y.; Arakawa, Y.; Tada, M. *Bioorg. Medi. Chem.* **2001**, *9*, 347.