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Anti-*Helicobacter pylori* Compounds from *Santalum album*

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Six new sesquiterpenes, (*Z*)-2β-hydroxy-14-hydro-β-santalol (**1**), (*Z*)-2α-hydroxy-albumol (**2**), 2*R*-(*Z*)-campherene-2,13-diol (**3**), (*Z*)-campherene-2β,13-diol (**4**), (*Z*)-7-hydroxynuciferol (**5**), and (*Z*)-1β-hydroxy-2-hydrolanceol (**6**), together with five known compounds, (*Z*)-α-santalol (**7**), (*Z*)-β-santalol (**8**), (*Z*)-lanceol (**9**), α-santaldiol (**10**), and β-santaldiol (**11**), were isolated from *Santalum album*, by using bioassay-guided fractionation for *Helicobacter pylori*. The structures were determined by extensive NMR studies. The absolute configuration of compound **3** was determined by a modified Mosher method. The crude extracts as well as the isolated compounds showed antibacterial activity against *H. pylori*. Especially, compounds **7** and **8** have strong anti-*H. pylori* activities against a clarithromycin-resistant strain (TS281) as well as other strains.

Helicobacter pylori may be the most common infectious disease worldwide. The prevalence of *H. pylori* infection worldwide is approximately 50%;[1] in Japan, it is extremely low at an early age, as in other developed countries, and subsequently shows a rapid increase until it reaches a plateau of about 70% at 50 years of age.[2] It is recognized as a major causal factor in chronic gastritis and peptic ulcer and a risk factor in the development of gastric cancer.[3] Treatment of the infection is mainly based on the use of triple therapies consisting of a combination of two antibiotics (clarithromycin, amoxicillin, and/or metronidazole) with a proton pump inhibitor. However, antibiotic-resistant strains of *H. pylori* have emerged and are becoming problematic throughout the world.[1,4−6] Infection by a clarithromycin- or metronidazole-resistant *H. pylori* strain is an important factor leading to treatment failure, as clarithromycin and metronidazole are highly recommended for the treatment. In Japan, clarithromycin-resistant *H. pylori* strains have increased rapidly since 1995, and there is apprehension that the effectiveness of clarithromycin-based *H. pylori* eradication therapies may soon be compromised.[6] Therefore, there is an urgent need for new chemotherapeutic agents having excellent activity against *H. pylori* for treatment and prevention.

The first and most important step to discover such agents is the identification of lead compounds from natural substances. In recent years, there have been a number of reports of aqueous garlic extracts being inhibitory to *H. pylori* in vitro.[7,8] Hence, we examined crude extracts of various spices for anti-*H. pylori* activity and found that, among the extracts examined, a MeOH extract of sandalwood was the most potent.

Sandalwood, *Santalum album* L., has been used since ancient times for religious purposes in incense, in fragrances, and as medicine. Various types of sandalwood trees grow in different countries of the world.[9]

Using an in vitro anti-*H. pylori* activity test to guide isolation, the MeOH extract of *S. album* was subjected to further fractionation and purification procedures to yield six new and five known compounds. In this paper, we describe their isolation, structural determination, and anti-*H. pylori* activities.

Results and Discussion

The anti-*H. pylori* activities of MeOH, EtOAc, *n*-BuOH, and H_2O extracts of 25 spices were assessed by the presence

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Table 1. Antibacterial Activity of Various Spices against *H. pylori* ATCC43504 Based on the Disk Method[a]

spice		inhibition zone in diameter (mm)			
		MeOH ext.	EtOAc ext.	BuOH ext.	H_2O ext.
allspice		NT	−	−	+
anise		NT	12.2	14.0	−
celery seed		NT	12.3	+	−
cinnamon		NT	17.3	+	−
clove		NT	+	9.4	−
coriander		+	+	+	−
cumin		NT	+	−	−
fennel		−	−	+	−
lemon balm		NT	−	+	−
mace		NT	20.7	20.0	−
marjoram		NT	11.1	+	+
mint		NT	+	+	−
mustard		−	−	NT	−
oregano		+	10.2	−	−
parsley		−	+	−	−
pepper	black	NT	16.2	17.2	−
	long	19.4	17.1	16.1	−
	white	NT	20.7	16.5	−
rosemary		+	11.0	10.6	−
sage		12.5	+	9.5	−
sandalwood		39.8	37.7	37.7	28.8
sesame	black	12.4	NT	NT	NT
	white	16.4	NT	NT	NT
staranis		9.1	10.4	−	−
thyme		10.0	16.5	+	−

[a] Amounts of extract tested were 100 mg/disk. +, smaller than 9 mm; −, no effect; NT, not tested.

Table 2. ^{13}C NMR Data for Compounds **1**−**9** ($CDCl_3$, δ_C)

carbon	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	52.2	48.9	50.4	49.3	136.2	69.0	27.4	46.8	133.8
2	81.3	83.4	77.4	79.7	128.9	38.9	19.5	166.2	120.7
3	46.2	42.2	38.8	39.9	124.7	27.4	31.5	44.8	31.4
4	47.6	46.6	42.0	41.9	144.7	43.6	38.2	44.7	39.8
5	24.0	26.2	28.0	27.1	124.7	27.4	31.0	23.7	28.3
6	23.2	25.5	26.1	34.4	128.9	38.9	19.5	29.7	30.7
7	34.2	40.9	51.3	50.0	74.9	153.9	45.9	37.1	153.9
8	38.1	42.7	32.8	33.6	43.8	34.9	35.0	41.5	35.0
9	24.7	22.6	23.8	23.3	22.6	26.4	22.9	23.2	26.4
10	129.5	129.3	129.1	129.4	128.4	128.1	129.5	129.0	128.1
11	134.0	133.9	134.0	133.9	134.6	134.5	133.7	133.9	134.5
12	21.8	21.4	21.3	21.4	21.4	21.3	21.2	21.2	21.3
13	61.7	61.4	61.5	61.3	61.5	61.6	61.6	61.6	61.6
14	22.3	19.5	16.6	16.8	21.0	31.4	17.5	99.7	23.4
15	19.9	16.7	13.4	11.4	30.8	107.6	10.6	22.6	107.5

or absence of inhibition zones and zone diameter based on the disk diffusion method. The results are presented in Table 1. Of 25 spices tested, 17, including peppers, showed significant but varied anti-*H. pylori* activity. The anti-*H. pylori* activity was found with EtOAc and *n*-BuOH extracts but not with H_2O extracts except that the H_2O extract of sandalwood showed potent activity. The maximal inhibition zone was shown by the MeOH extract of sandalwood. For this reason, we focused our further screening on the MeOH extract of sandalwood.

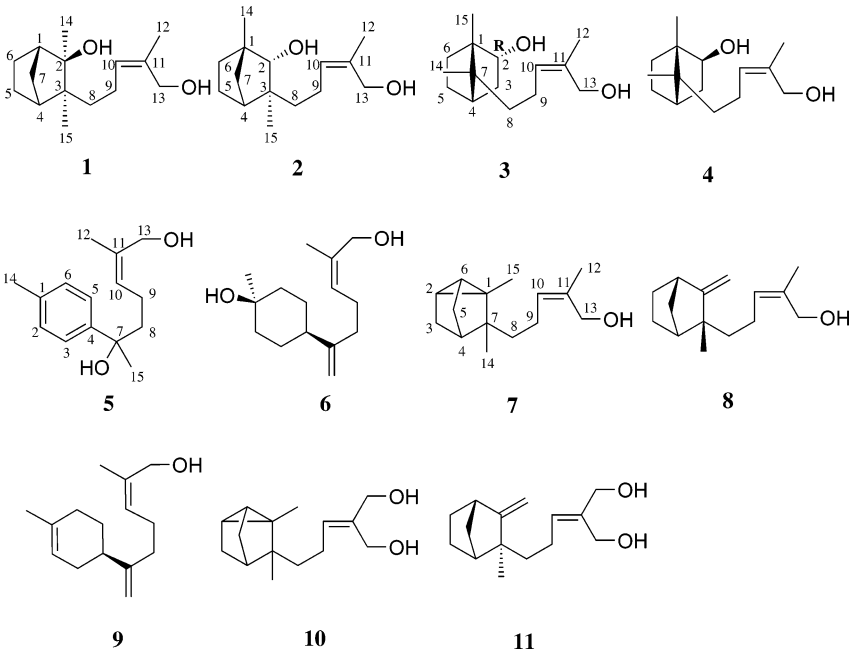
Using an in vitro anti-*H. pylori* activity test to guide isolation, the MeOH extract of *S. album* was subjected to further fractionation and purification procedures to yield six new and five known compounds.

The air-dried crushed heartwood of *S. album* L. was extracted with MeOH. After the solvent was removed, the residue was partitioned between H_2O and EtOAc. Bioassay-guided purification of the active compounds from the EtOAc-soluble fraction led to the isolation of six new (**1**−**6**) and five known (**7**−**11**) compounds. Compounds **9**−**11** were identified as (*Z*)-lanceol (**9**), α-santaldiol (**10**), and *β*-santaldiol (**11**), by analysis of the NOESY spectrum and comparing their spectroscopic data with those reported.[10,11]

Compounds **7** and **8** are known as (*Z*)-α-santalol and (*Z*)-*β*-santalol, respectively, but complete assignment of their NMR data has not been done. Therefore, we measured and assigned the data of **7** and **8** by analysis 1D and 2D NMR and comparison with the reported data[10] of **10** and **11**. On comparing the ^{13}C NMR data (Table 2) of **7** and **8** with the reported data[10] of **10** and **11**, **7** and **8** were found to be similar to **10** and **11**, respectively, except for the presence of 12-CH_2OH in **10** and **11** compared to 12-CH_3 in **7** and **8**. In the NOESY spectrum of **7**, the correlation between H-10 and H-12 was observed. In the NOESY spectrum of **8**, the correlations between H-5α and H-15 and between H-10 and H-12 were observed. From these results, **7** and **8** were defined as (*Z*)-α-santalol and (*Z*)-*β*-santalol, respectively (Chart 1).

Compound **1** showed an $[M + Na]^+$ ion peak at *m/z* 261.1821 in the HRFABMS, suggesting a molecular formula of $C_{15}H_{26}O_2$. The ^{13}C NMR spectrum showed signals due to three methyl carbons (δ_C 22.3, 21.8, 19.9), six methylene carbons (δ_C 61.7, 38.1, 34.2, 24.7, 24.0, 23.2), three methine carbons (δ_C 129.5, 52.2, 47.6), and three quaternary carbons (δ_C 134.0, 81.3, 46.2). The ^1H NMR spectrum also showed the presence of three methyls [δ_H

Chart 1



1.80 (3H, s), 1.21 (3H, s), 0.89 (3H, s)], one olefinic proton [δ_H 5.35 (1H, brs)], and one oxygenated methylene proton [δ_H 4.21 (1H, d, J = 11.6), 4.00 (1H, d, J = 11.6)]. These results showed that **1** had the same number of methyl, methylene, methine, and quaternary carbons as shown in **8** and were identical except for the substitiution of an olefinic methylene and a quaternary carbon in **8** in place of a methyl and oxygenated quaternary carbon in **1**. The ^{13}CNMR data of **1** were similar to those of **8**, except for the chemical shifts of C-1, C-2, C-6, and C-14. This difference was assumed to be attributed to the fact that compound **1** is a hydrated derivative of **8** at C-2. In the HMBC spectrum of **1**, the correlation of the proton signals at δ_H 1.21 (H-14) with the carbon signals at δ_C 46.2 (C-3), 52.2 (C-1), and 81.3 (C-2), and the proton signal at δ_H 0.89 (H-15) with the carbon signals at δ_C 38.1 (C-8), 46.2 (C-3), 47.6 (C-4), and 81.3 (C-2), indicated a C-2 hydroxy group. The relative configuration of **1** was determined on the basis of the NOESY spectrum. The correlations between H-15 and H-5α, between H-15 and H-14, between H-14 and H-6α, between H-6β and H-7, and between H-7 and H-8 indicated that the orientations of 2-OH and 7-CH_2 are β, while that of 15-CH_3 is α. The NOE correlations between H-9 and H-13 and between H-10 and H-12 indicated a *Z* configuration for the double bond (Figure 1). These results confirmed that **1** is (*Z*)-2β-hydroxy-14-hydro-β-santalol.

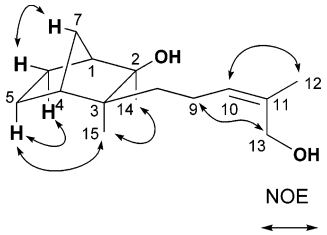


Figure 1. NOE correlations of **1**.

Compound **2** showed an $[M + Na]^+$ ion peak at *m/z* 261.1852 in the HRFABMS, suggesting a molecular formula of $C_{15}H_{26}O_2$. The ^{13}C NMR spectrum showed signals due to three methyl carbons (δ_C 21.4, 19.5, 16.7), one oxygenated methylene carbon (δ_C 61.4), one olefinic methine carbon (δ_C 129.3), one oxygenated methine carbon (δ_C 83.4), one quaternary olefinic carbon (δ_C 133.9), five methylenes, one methine, and two quaternary carbons. The ^{1}H NMR spectrum also showed the presence of three methyls [δ_H 1.80 (3H, s), 1.09 (3H, s), 0.71 (3H, s)], one olefinic proton [δ_H 5.30 (1H, t, J = 7.4)], one oxygenated methylene proton [δ_H 4.16 (1H, d, J = 11.6), 4.09 (1H, d, J = 11.6)], and one oxygenated methine proton [δ_H 3.30 (1H, s)]. These results showed that **2** had the same number of methyl, methylene, methine, and quaternary carbons as shown in **1**, but one methine in **1** was changed to one oxygenated methine in **2**. The chemical shifts of the side chain protons of both compounds were almost identical. The difference between the two compounds was most likely due to the position of one of the methyl groups of the bicyclic ring system. In the HMBC spectrum of **2**, the correlation of the proton signal at δ_H 1.06 (H-14) with δ_C 25.5 (C-6), 40.9 (C-7), 48.9 (C-1), and 83.4 (C-2), the proton signal at δ_H 0.71 (H-15) with δ_C 42.2 (C-3), 42.7 (C-8), 46.6 (C-4), and 83.4 (C-2), and the proton signal at δ_H 1.76 (H-4) with δ_C 25.5 (C-6), 48.9 (C-1), and 83.4 (C-2) indicated that methyl (C-14) and hydroxy should be attached to C-1 and C-2, respectively. In the NOESY spectrum of **2**, correlations between H-15 and H-5α, between H-5β and H-7, and between H-7 and H-2 indicated that the orientations of 2-OH and 15-CH_3 are α, while that of 7-CH_2 is β. The NOE correlations between H-9 and H-13 and between H-10 and H-12 confirmed a *Z*-configured double bond (Figure 2). From these results, the structure of **2** was determined as (*Z*)-2α-hydroxyalbumol.

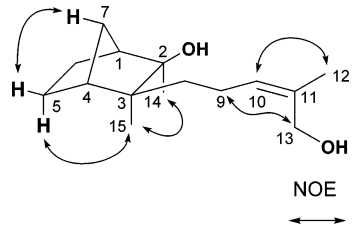


Figure 2. NOE correlations of **2**.

The ^{1}H and ^{13}C NMR data of **3** ($C_{15}H_{26}O_2$) were very similar to that of (*E*)-campherene-2α,13-diol[12] except for

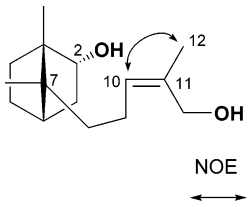
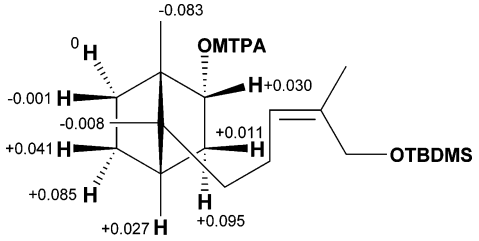


Figure 3. NOE correlations of **3**.



$f\not{c}\delta = \delta S - \delta R$: ppm

Figure 4. $\Delta\delta$ values of (*S*)- and (*R*)-MTPA esters of **3a**.

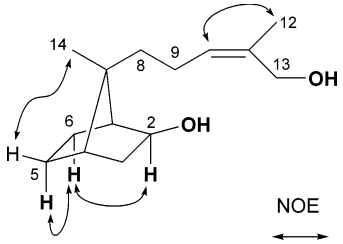


Figure 5. NOE correlations of **5**.

the data of the side chain double bond. The relative configuration was established by comparison of reference data.[12] NOE correlations were observed between H-10 and H-12, confirming the *Z* configuration for the double bond (Figure 3). To determine the absolute configuration of **3**, it was protected with *tert*-butyldimethylsilyl (TBDMS) to give **3a**. The (*S*)-MTPA ester (**3a*S***) was obtained by treatment of **3a** with (*S*)-MTPA, and the (*R*)-MTPA ester (**3a*R***) by treatment with (*R*)-MTPA chloride. Thus, the 2*R* absolute configuration could be assigned on the basis of the $\Delta\delta$ values ($\Delta\delta = \delta S - \delta R$)[13,14] (Figure 4). These results confirmed that **3** is 2*R*-(*Z*)-campherene-2,13-diol.

Compound **4** showed a molecular ion at *m/z* 238.1919 in the HREIMS, suggesting a molecular formula of $C_{15}H_{26}O_2$. The ^{1}H NMR spectrum of **4** was similar to that of **3**, except for the H-2 proton signals [**3**: δ_H 4.08 (1H, brd, J = 10.4), **4**: δ_H 3.65 (1H, dd, J = 8.0, 3.6)] and H-13 [**3**: δ_H 4.15 (2H, s), **4**: δ_H 4.15, 4.09 (each 1H, d, J = 11.8)]. The ^{13}C NMR spectrum of **4** was similar to that of **3**, except for the chemical shifts of C-2, -6, and -15 (Table 2). The HSQC and HMBC data indicated that **3** and **4** had the same planar structure. In the NOESY spectrum, correlations between 14-CH_3 and H-5β, between H-5α and H-6α, and between H-6α and H-2 indicated β-orientations for 2-OH and 7-CH_2, while correlations between H-10 and H-12 (Figure 5) confirmed the *Z*-configured double bond. These results confirmed that **4** is (*Z*)-campherene-2β,13-diol.

The ^{13}C NMR spectrum of **5** ($C_{15}H_{22}O_2$) showed signals due to three methyl carbons (δ_C 30.8, 21.4, 21.0), three methylene carbons (δ_C 61.5, 43.8, 22.6), five methine carbons (δ_C 128.9 × 2, 128.4, 124.7 × 2), and four quaternary carbons (δ_C 144.7, 136.2, 134.6, 74.9). The ^{1}H NMR spectrum also showed the presence of three methyls [δ_H 2.36 (3H, s), 1.76 (3H, s), 1.55 (3H, s)], one olefinic proton [δ_H 5.28 (1H, t, J = 7.0)], and a 1,4-disubstituted benzene ring [δ_H 7.32, 7.17 (both 2H, d, J = 8.0)].

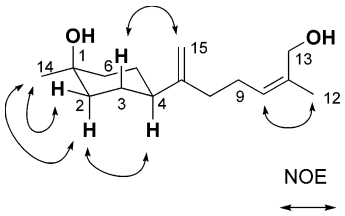


Figure 6. NOE correlations of **6**.

Table 3. Anti-*H. pylori* Activities of Compounds Isolated from *Santalum album* Based on the Disk-Diffusion Method

	inhibition zone in diameter (mm)	
compound (100 μg/disk)	*H. pylori* ATCC43504	*H. pylori* SS-1
1	20.1	16.8
2	27.6	15.5
3	27.1	11.1
4	28.9	22.8
5	24.9	11.2
6	8.9	(
7	21.6	11.5
8	27.2	13.5
9	11.9	10.6
10	38.7	25.9
11	21.1	10.4
AMPC[a]	35.3	20.3

[a] AMPC, amoxicillin (250 ng/disk), was used as positive control.

The ^{13}C NMR chemical shifts (C-9−C-13) of compound **5** were very similar to those of compound **9**. In the HMBC spectrum of **5**, the proton signals at δ_H 1.55 (H-15) were correlated with the carbon signals at δ_C 43.8 (C-8), 74.9 (C-7), and 144.7 (C-4), and the proton signal at δ_H 2.07 (H-9) with δ_C 43.8 (C-8), 74.9 (C-7), 128.4 (C-10), and 134.6 (C-11). In the NOESY spectrum of **5**, correlations between H-10 and H-12, and H-9 and H-13, again confirmed the *Z*-configuration for the double bond. These results indicated that compound **5** is (*Z*)-5-hydroxynuciferol.

The ^{13}C NMR data of compounds **6** ($C_{15}H_{26}O_2$) and **9** were similar except for the chemical shifts of C-1−C-6. From the molecular formula and chemical shifts of **6** its structure was assumed to be a hydrated analogue of the **9** ring double bond. In the NOESY spectrum of **6**, correlations between H-14 and H-2α, between H-14 and H-2β, between H-4 and H-2α, and between H-15 and H-3β confirmed the β-axial orientations of 1-OH and H-4. The double bond has a *Z* configuration, on the basis of the NOE correlation between H-10 and H-12 (Figure 6). These results confirmed that **6** is (*Z*)-1β-hydroxy-2- hydrolanceol.

The anti-*H. pylori* activities of isolated compounds (**1**−**11**) based on the disk diffusion method are shown in Table 3. They have significant but varied activity against *H. pylori*. Except for compounds **6** and **9** the remaining analogues strongly inhibited *H. pylori* growth, with compound **10** being the most potent. The MIC values of sesquiterpenes **7**, **8**, and **9** are shown in Table 4. Anti-*H. pylori* potency was considerably greater (2- to 8-fold-lower MICs) with compounds **7** and **8** than with compound **9**. Compared to amoxicillin and clarithromycin, these compounds showed inhibitory effects on the growth of sensitive strains of *H. pylori* at a much higher concentration. However, it should be noted that compounds **7** and **8** were more potent than clarithromycin against the antibiotic-resistant strain TS281.

From these results, it is obvious that compounds from *S. album* could potentially contribute to the development of new chemotherapeutic agents against clarithromycin-resistant strains of *H. pylori*.

Table 4. MIC Values of Compounds **7**, **8**, and **9** against *H. pylori*

	MIC (μg/mL)				
H. pylori strain	amoxicillin	clarithromycin	**7**	**8**	**9**
ATCC43504	0.06	≤0.06	15.6	7.8	62.5
SS1	0.25	≤0.06	31.3	31.3	125
Sa-1	0.06	≤0.06	7.8	7.8	31.3
Sa-2	0.06	≤0.06	31.3	7.8	62.5
Sa-3	0.25	≤0.06	7.8	7.8	31.3
TPH30	0.25	≤0.06	15.6	7.8	62.5
TS281	0.5	32	15.6	7.8	62.5
TS648	0.13	2	7.8	7.8	31.3

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a JASCO Fourier transform infrared spectrometer (FT/IR-420). NMR (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR, both used TMS as internal standard) were measured on a Bruker AM 400 spectrometer, and MS on a JEOL JMSD-300 instrument. Column chromatography: silica gel 60 (Merck) and Toyopearl HW-40 (TOSHO). HPLC: silica gel (YMC-pack SIL-06 SH-043-5-06, 250 × 20 mm, Hibar RT 250-25 Si 60). Gel-permeation chromatography (GPC): Shodex H-2001, 2002, $CHCl_3$; Asahipak GS-310 2G, MeOH.

Plant Material for Screening. Spices were purchased from commercial sources and identified by Dr. K. Murakami, Graduate School of Pharmaceutical Sciences, University of Tokushima. Voucher specimens (allspice: 00578, anise: 00579, Celery seed: 00575, cinnamon: 00576, clove: 00573, coriander: 00563, cumin: 00572, fennel: 00567, lemon balm: 00574, mace: 00570, marjoram: 00571, mint: 00561, mustard: 00556, oregano: 00564, parsley: 00560, black pepper: 00550, long pepper: 00552, white pepper: 00569, rosemary: 00557, sage: 00558, sandalwood: 00551, black sesame: 00566, white sesame: 00565, staranis: 00562, thyme: 00559) were deposited in the herbarian of the Graduate School of Pharmaceutical Sciences, University of Tokushima.

Extraction of Spices. The sample (60 g) of spices was extracted with hot MeOH for 2 h (×2). The MeOH solution was concentrated in vacuo, and the resulting concentrate was partitioned between EtOAc, *n*-BuOH, and H_2O. Their extracts were screened for antimicrobial activity against *H.* pylori. Results are shown in Table 1.

Plant Material. The heartwood of *S. album*. (2.8 kg) was purchased from Alps Pharmaceutical Ind. Co., Ltd., Japan. A voucher specimen (No. 00551) was deposited in the Herbarium of Faculty of Pharmaceutical Sciences, University of Tokushima, Japan.

Extraction and Isolation. The heartwood of *S. album* (2.8 kg) was extracted using MeOH at 70 °C. The extract was filtered and concentrated in vacuo, and the resulting concentrate (205 g) was successively partitioned between EtOAc, BuOH, and H_2O. The major antibacterial activity against *Helicobacter pyrori* was found in the EtOAc extract. The EtOAc extract was subjected to silica gel column chromatography using different solvents of increasing polality (*n*-hexane−EtOAc; EtOAc−MeOH) to give nine fractions (1−9). A part (300 mg) of fraction 2 (50.5 g) was subjected to gel-permeation chromatography (GPC) ($CHCl_3$) to give eight fractions (2.1−2.8). Fraction 2.6 (25 mg) was subjected to HPLC (silica gel, *n*-hexane−EtOAc, 4:1) to give **9** (8 mg). Fraction 2.7 (151 mg) was subjected to HPLC (silica gel, *n*-hexane−EtOAc, 4:1) to give **7** (60 mg) and **8** (38 mg). Fraction 5 (25.1 g) was subjected to silica gel column chromatography using different solvents of increasing polarity (*n*-hexane−EtOAc; EtOAc−MeOH) to give four fractions (5.1−5.4). The major antibacterial activity was found in fraction 5.3 (12.0 g). A part (3.5 g) of fraction 5.3 (12.0 g) was subjected to Toyopearl HW-40 column chromatography ($CHCl_3$−MeOH, 2:1) to give two fractions (5.3.1, 5.3.2). Fraction 5.3.2 (1.9 g) was subjected to silica gel column chromatography using different solvents of increasing polarity (*n*-hexane−EtOAc) to give three fractions (5.3.2.1−5.3.2.3). Fraction 5.3.2.1 (353 mg) was subjected to HPLC (silica gel, *n*-hexane−EtOAc, 2:1) to give **1** (61 mg) and **2** (8 mg). Fraction 5.3.2.2 (704 mg) was subjected to HPLC (silica gel, *n*-hexane−EtOAc, 1:1) to give **3** (65 mg), **4** (12 mg), **5** (3 mg), and **6** (4 mg). Fraction 5.3.2.3 (634 mg) was subjected to HPLC (silica gel, *n*-hexane−EtOAc, 1:2) to give **9** (95 mg) and **10** (10 mg.).

(*Z*)-2*β*-Hydroxy-14-hydro-*β*-santalol (1): colorless oil; $[\alpha]_D$ −8.5° (*c* 0.9, $CHCl_3$); IR (KBr) ν_{max} 3398, 2951, 2877, 1455, 1380 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ_H 5.35 (1H, brs, H-10), 4.21 (1H, d, *J* = 11.6, H-13), 4.00 (1H, d, *J* = 11.6, H-13), 2.14(1H, m, H-9), 1.80 (3H, s, H-12), 1.57 (1H, m, H-5α), 1.21 (3H, s, H-14), 0.89 (3H, s, H-15); ^{13}C NMR data, see Table 1; HRFABMS *m/z* 261.1821 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_2Na$, 261.1830).

(*Z*)-2α-Hydroxyalbumol (2): colorless oil; $[\alpha]_D$ −2.3° (*c* 1.9, $CHCl_3$); IR (KBr) ν_{max} 3375, 2949, 2872, 1455, 1376 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ_H 5.30 (1H, t, *J* = 7.4, H-10), 4.16 (1H, d, *J* = 11.6, H-13), 4.09 (1H, d, *J* = 11.6, H-13), 3.30 (1H, s, H-2*β*), 2.07 (1H, m, H-9), 1.80 (3H, s, H-12), 1.76 (1H, m, H-4), 1.66 (1H, m, H-5α), 1.42 (1H, m, H-7), 1.09 (3H, s, H-14), 0.71 (3H, s, H-15); ^{13}C NMR data, see Table 1; HRFABMS *m/z* 261.1852 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_2Na$, 261.1830).

2*R*-(*Z*)-Campherene-2,13-diol (3): colorless oil; $[\alpha]_D$ −11.5° (*c* 4.0, $CHCl_3$); IR (KBr) ν_{max} 3366, 2951, 2875, 1453, 1378 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ_H 5.32 (1H, t, *J* = 7.2, H-10), 4.15 (2H, s, H-13), 4.08 (1H, brd, *J* = 10.4, H-2), 1.80 (3H, s, H-12), 0.89 (3H, s, H-14), 086 (3H, s, H-15); ^{13}C NMR data, see Table 1; HREIMS *m/z* 238.1941 $[M]^+$ (calcd for $C_{15}H_{26}O_2$, 238.1933).

(*Z*)-Campherene-2*β*,13-diol (4): colorless oil; $[\alpha]_D$ −1.1° (*c* 1.4, $CHCl_3$); IR (KBr) ν_{max} 3323, 2961, 2873, 1453, 1375 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ_H 5.35 (1H, t, *J* = 7.4, H-10), 4.15 (1H, d, *J* = 11.8, H-13), 4.09 (1H, d, *J* = 11.8, H-13), 3.65 (1H, dd, *J* = 8.0, 3.6, H-2α), 1.58 (3H, s, H-12), 1.02 (1H, m, H-5α), 0.92 (1H, m, H-6α), 0.82 (3H, s, H-15), 0.66 (3H, s, H-14); ^{13}C NMR data, see Table 1; HREIMS *m/z* 238.1919 $[M]^+$ (calcd for $C_{15}H_{26}O_2$, 238.1933).

(*Z*)-7-Hydroxynuciferol (5): colorless oil; $[\alpha]_D$ −5.0° (*c* 0.4, $CHCl_3$); IR (KBr) ν_{max} 3375, 2927, 2872, 1453, 1374 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ_H 7.32 (2H, d, *J* = 8.0, H-3, 5), 7.17 (2H, d, *J* = 8.0, H-2, 6), 5.28 (1H, t, *J* = 7.0, H-10), 4.01 (2H, s, H-13), 2.36 (3H, s, H-14), 2.07 (1H, m, H-9), 1.76 (3H, s, H-12), 1.55 (3H, s, H-15); ^{13}C NMR data, see Table 1; HREIMS *m/z* 234.1648 $[M]^+$ (calcd for $C_{15}H_{22}O_2$, 234.1620).

(*Z*)-1*β*-Hydroxy-2-hydrolanceol (6): colorless oil; $[\alpha]_D$ −3.0° (*c* 0.5, $CHCl_3$); IR (KBr) ν_{max} 3373, 2928, 2859, 1447, 1375 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ_H 5.33 (1H, t, *J* = 6.8, H-10), 4.82 (1H, s, H-15), 4.72 (1H, s, H-15), 4.14 (2H, s, H-13), 1.82 (1H, m, H-4), 1.81 (3H, s, H-12), 1.72 (2H, m, H-2*β*, 6*β*), 1.60 (2H, m, H-3*β*, 5*β*), 1.56 (2H, m, H-3α, 5α), 1.45 (2H, m, H-2α, 6α), 1.25 (3H, s, H-14); ^{13}C NMR data, see Table 1; HREIMS *m/z* 220.1826 $[M - H_2O]^+$ (calcd for $C_{15}H_{24}O$, 220.1827).

Preparation of the 13-*O*-TBDMS Ester of 3. To compound **3** (4.8 mg) was added TBDMS chloride (15.1 mg) in CH_2Cl_2 (2 mL) followed by triethylamine (10.2 mg) and DMAP (1.0 mg). After stirring overnight at room temperature, the mixture was washed with saturated $NaHCO_3$ and saturated NH_4Cl, dried over Na_2SO_4, and concentrated. The residue was purified by PTLC, giving 3.5 mg of the TBDMS derivative **3a**.

Preparation of (*S*)-MTPA Ester (3a*S*). A solution of the TBDMS derivative (**3a**) (2.5 mg, 0.007 mmol) in CH_2Cl_2 (2 mL) was added to (*S*)-MTPA (8.3 mg), EDC (ethylenedichloride) (2.5 mg), triethylamine (7.9 mg), and DMAP (2 mg). After the solution was stirred for 1 day at room temperature, the mixture was washed with 10% citric acid and saturated $NaHCO_3$, water, and brine and dried over Na_2SO_4. The organic layer was concentrated, and the residue was purified by PTLC to give the ester **3a*S*** (2.0 mg).

Preparation of (*R*)-MTPA Ester (3a*R*). A solution of the derivative **3a** (2.1 mg) in pyridine was added to (*R*)-MTPA chloride (15.2 mg), and the solution was allowed to stand at room temperature overnight. 3-(Dimethylamino)propylamine (6.1 mg) was added, and after 10 min the solvent was evaporated. The residue was purified by PTLC to give ester **3a*R*** (3.0 mg).

3a*S*: ^{1}H NMR ($CDCl_3$) δ_H 5.090 (H-2), 1.052 (H-3α), 2.278 (H-2*β*), 1.802 (H-4), 1.137 (H-5α), 1.629 (H-5*β*), 1.748 (H-6α), 1.210 (H-6*β*), 1.286 (H-8), 1.086 (H-8), 2.048 (H-9), 1.825 (H-9), 5.090 (H-10), 1.662 (H-12), 4.070 (H-13), 0.808 (H-14), 0.720 (H-15).

3a*R*: ^{1}H NMR ($CDCl_3$) δ_H 5.060 (H-2), 0.956 (H-3α), 2.267 (H-2*β*), 1.775 (H-4), 1.052 (H-5α), 1.588 (H-5*β*), 1.748 (H-6α), 1.211 (H-6*β*), 1.288 (H-8), 1.081 (H-8), 2.047 (H-9), 1.825 (H-9), 5.131 (H-10), 1.662 (H-12), 14.069 (H-13), 0.816 (H-14), 0.803 (H-15).

Bacterial Strains and Cultures. *H. pylori* ATCC43504, SS1, and three clinical isolates (Sa-1, Sa-2, and Sa-3) were donated by Prof. Dr. Keiji Oguma (Departments of Pathology, Bacteriology, and Medicine, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan). Clarithromycin-resistant strains TS281 and TS648 were obtained from Prof. Dr. Masanori Sasatsu (Department of Microbiology, School of Pharmacy, Tokyo University of Pharmacy and Life Science, Tokyo, Japan). A clinical strain, TPH30, was obtained from the Department of Clinical Laboratories, Tokushima Prefectural Central Hospital (Tokushima, Japan). Until processed, the strains were stored at −80 °C in Brucella broth (Difco Laboratories, Detroit, MI) supplemented with 20% glycerol.

Antibacterial Screening. The disk-diffusion method was used to screen the anti-*H. pylori* activity of the extracts. Sample solutions (10 mg/mL) were prepared by dissolving the extracts in DMSO (Kanto Chemical Co., Inc., Tokyo, Japan). Positive control used the antibiotic amoxicillin (Sigma Chemical Co., St. Louis, MO).

H. pylori (ATCC43504) was cultured for 4 days at 37 °C in Brucella broth containing 5% horse serum (Bio Whittaker, Walkersville, MD) under micro-aerophilic conditions using a disposable O_2 absorbing and CO_2 generating agent, Anaero-Pack Helico (Mitsubishi Gas Chemical Co., Inc., Tokyo, Japan), with humidity. The culture was then diluted and adjusted to about 1×10^7 CFU/mL with the fresh medium and was uniformly spread with a cotton swab onto the ISO-SENSI test agar (Oxoid Ltd., Basingstoke, Hampshire, England) containing 10% horse blood (Nippon Biotest Laboratories Inc., Tokyo, Japan). Sterile blank disks (Whatman AA Discs, 6 mm, Whatman International Ltd., Maidstone, England) were placed on the agar surface. Then, 10 μL of the sample solutions was transfused onto the disks. After 4 days' incubation at 37 °C under the micro-aerophilic conditions with humidity, the plates were screened for growth inhibition zones.

MIC Determination. The MICs of the chemicals and antibiotics were determined by the 2-fold plate-dilution method using Mueller-Hinton medium supplemented with 5% sheep blood (Nippon Biotest Laboratories Inc.). Bacterial suspensions equivalent to a 2.0 MacFarland standard (containing 1×10^8 CFU/mL) were prepared in saline from a 72 h subculture from a blood agar plate, and the bacteria (5 μL per spot) were applied with an inoculator onto the surfaces of 10 mm agar layers. The plates were read after 4 days' incubation at 37 °C under the micro-aerophilic conditions with humidity.

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