CJ-12,954 and Its Congeners, New Anti-*Helicobacter pylori* Compounds Produced by *Phanerochaete velutina*: Fermentation, Isolation, Structural Elucidation and Biological Activities

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Seven new phthalide compounds with anti-*Helicobacter pylori* activities were isolated from the basidiomycete *Phanerochaete velutina* CL6387. The two most potent phthalide compounds, CJ-12,954 and CJ-13,014, have MICs of 5 ng/ml. The structure-activity relationship shows that the presence of a spiroketal part in addition to the phthalide part, greatly enhances the activity. The phthalide compounds appear to be specific for *H. pylori*, since they did not show antibacterial activities when tested against a panel of other microorganisms.

Gastric and duodenal ulcers affect a significant portion of the human population worldwide. Many recent studies have shown a relation between the presence of the microaerophilic Gram-negative bacterium *Helicobacter pylori*, which appears to live beneath the mucus layer of the stomach, and gastric and duodenal ulcers[1,2]. Thus, therapy to eliminate *H. pylori* from the gastroduodenal tract would remove the root cause of gastric and duodenal ulcers. Current therapy typically relies upon co-administration of one or two broad-spectrum antibiotics combined with a proton pump inhibitor or a bismuth salt. However, even triple therapy is not entirely successful in achieving long term eradication of *H. pylori*. Follow-up of patients cleared of *H. pylori* infection has shown that relapse (rather than reinfection) is a problem. Treatment of *H. pylori*-associated gastric disorders with antibacterial agents given by conventional dosing regimens thus requires a prolonged course of therapy to be effective[1,2]. However, long-term treatment with current therapies is not recommended in view of side-effects, the build-up of drug resistance and, in particular, the inherent toxicity risk associated with a bismuth salt. Accordingly, there is a need for a safe and effective treatment with a compound having excellent anti-*H. pylori* activity. In a screening program designed to discover such compounds from microbial secondary metabolites, the basidiomycete *Phanerochaete velutina* was found to produce new phthalide compounds with anti-*Helicobacter pylori* activity. In this paper, we report the fermentation, isolation, structural elucidation and biological activities of these phthalide compounds.

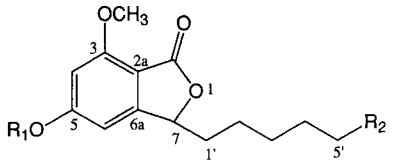
Producing Strain

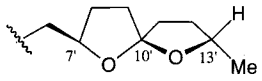
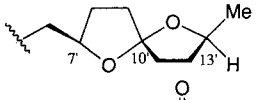
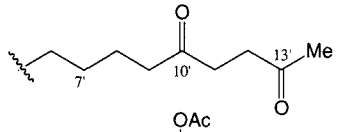
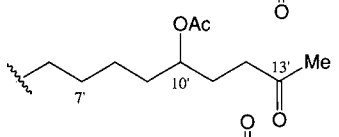
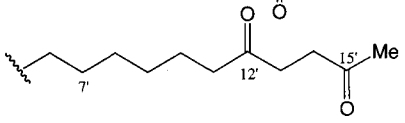
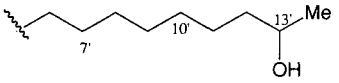
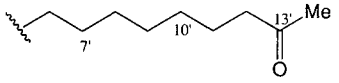
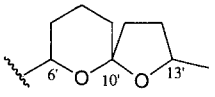
The producing strain, the basidiomycete *Phanerochaete velutina* CL6387, was obtained from the Forest Products Laboratory of the United States Department of Agriculture (Madison, Wisconsin) and deposited as FERM BP-4787 at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology (Tsukuba, Japan). The taxonomical properties of this strain have been reported by BURDSALL[3].

Fermentation

Phanerochaete velutina CL6387 was maintained on plates of malt agar medium (malt extract 2.5% and agar 1.5%). A cell suspension from the plate was used to inoculate 50-ml tubes containing 10 ml of Medium-1 (glucose 2%, malt extract 2%, yeast extract 1.8%, maltose 2.4% and agar 0.1%, pH 5.5). The tubes were incubated at 28°C on a shaker at 250 rpm for 14 days. The first seed culture (2 ml) was transferred to two 500-ml flasks containing 100 ml of Medium-1. The flasks were incubated at 28°C for 7 days. These flasks were used to inoculate 7.5 ml each into twelve 500-ml flasks containing 150 ml of Medium-1. These seed cultures in the 12 flasks were combined and used to inoculate 100 ml into each of twelve 6-liter fermentation vessels containing 600 ml

Fig. 1. Structures and helicobactericidal activities of the new phthalides **1**~**7** and spirolaxine (**8**).



	R_1	R_2	Activity (μg/disk that gives a 15 mm zone)
CJ-12,954 (**1**)	Me		0.02
CJ-13,014 (**2**)	Me		0.02
CJ-13,015 (**3**)	Me		2
CJ-13,102 (**4**)	Me		0.5
CJ-13,103 (**5**)	Me		50
CJ-13,104 (**6**)	Me		500
CJ-13,108 (**7**)	Me		10
Spirolaxine (**8**)	H		0.01

of Medium-1 and 300 g of wheat bran. Incubation was carried out at 28°C for 21 days.

Isolation of Phthalide Compounds

To each of the twelve 6-liter fermentation vessels, 2 liters of ethanol were added. After the combined broth was filtered, the filtrate was concentrated to aqueous solution (6 liters), and extracted with ethyl acetate (5 liters × 3). The extract was dried over anhydrous Na_2SO_4 and evaporated to afford an oily residue (84.4 g). The oily residue was loaded on a silica gel column (850 ml, Merck Kieselgel 60, 230~400 mesh) and compounds were eluted stepwise with 3 liters of ethyl acetate - hexane (1 : 3), followed by 3 liters of ethyl acetate - hexane (1 : 1). Fractions showing activity were applied separately to a Sephadex LH-20 (Pharmacia) column and eluted with methanol. Fractions containing phthalide compounds were further applied to a preparative HPLC (Chemcosorb 5ODS-UH, 20 × 250 mm) and eluted with acetonitrile - water (13 : 7). The eluted peaks showing activity were collected to yield the compounds CJ-12,954 (**1**, 15.2 mg), CJ-13,014 (**2**, 14.3 mg), CJ-13,015 (**3**, 7.8 mg), CJ-13,102 (**4**, 21.1 mg), CJ-13,103 (**5**, 4.0 mg), CJ-13,104 (**6**, 14.1 mg) and CJ-13,108 (**7**, 54.0 mg) (Fig. 1). For analysis, the 7 compounds were separated by HPLC (YMC-Pack ODS-AM, 5 μm, 4.6 × 150 mm) and eluted with methanol - water (3 : 1) at a flow rate of 1 ml/minute (42°C). The retention times (minute) of the separated compounds were 6.8 (**1**), 7.2 (**2**), 4.7 (**3**), 7.3 (**4**), 8.5 (**5**), 16.3 (**6**) and 14.6 (**7**).

Structural Elucidation

Structures of CJ-12,954 (1) and CJ-13,014 (2) (Fig. 2)

The molecular formula of **1** was determined to be $C_{24}H_{34}O_6$ by HREI-MS (*m/z* 418.2355, Δ +0.1 mmu), which corresponds to the number of protons and carbons observed by NMR. ^{1}H NMR data (in $CDCl_3$) indicated a total of 34 protons: 2 *meta*-coupled aromatic methines at 6.39 and 6.38 ppm (6.64 and 6.57 ppm coupled with 1.6 Hz in CD_3OD), 3 oxymethines at 5.27, 4.08 and 3.90 ppm, 2 aromatic methoxy groups at 3.93 and 3.87 ppm, 1 methyl doublet at 1.27 ppm, and 20 aliphatic protons between 2.05 and 1.20 ppm. One of the oxymethines at 5.27 ppm apparently attaches to an esteric oxygen. Because there are no D_2O-exchangeable protons observed, other oxygen-bearing signals should form ether. COSY and NOE difference experiments in $CDCl_3$ and CD_3OD revealed 3 fragments: 1,3-dimethoxy-5,6-disubstituted benzene which has $-CH(OCO-)CH_2-$ substitution at C-5 position, $-CH_2CH(O-)CH_2-$ and $-CH_2CH(O-)CH_3$. ^{13}C NMR data showed a total of 24 resonances (Table 1). DEPT spectra revealed 1 carbonyl group (168.5 ppm), 6 aromatic signals, 1 ketal singlet (114.2 ppm), 3 oxymethines (79.9 × 2, 75.8 ppm), 2 methoxy groups, 10 sp^3 triplets and 1 methyl group. From its chemical shift, the carbonyl group is most likely esteric and conjugated to an aromatic ring. The 6 aromatic signals are strongly polarized and separated into two regions on their chemical shifts (a group of singlets at 166.6, 159.6 and 155.2 ppm, and a group of a singlet at 106.9 ppm and doublets at 98.6 and 97.3 ppm), which would be expected to be a 1-carbonyl-2,4-dioxy-6-substituted benzene. These facts together with the benzene fragment from ^{1}H NMR suppose 3,5-dimethoxyphthalide as a partial structure of **1**. Also, the chemical shift of ketal carbon and two oxymethine fragments from ^{1}H NMR suggest the 1,6-dioxaspiro[4,4]nonane system. Since the remaining elements are only methylenes with no heteroatom substitution, the whole structure is proposed to be 3,5-dimethoxyphthalide extended with a straight alkyl chain having a 1,6-dioxaspiro-[4,4]nonane system at the end.

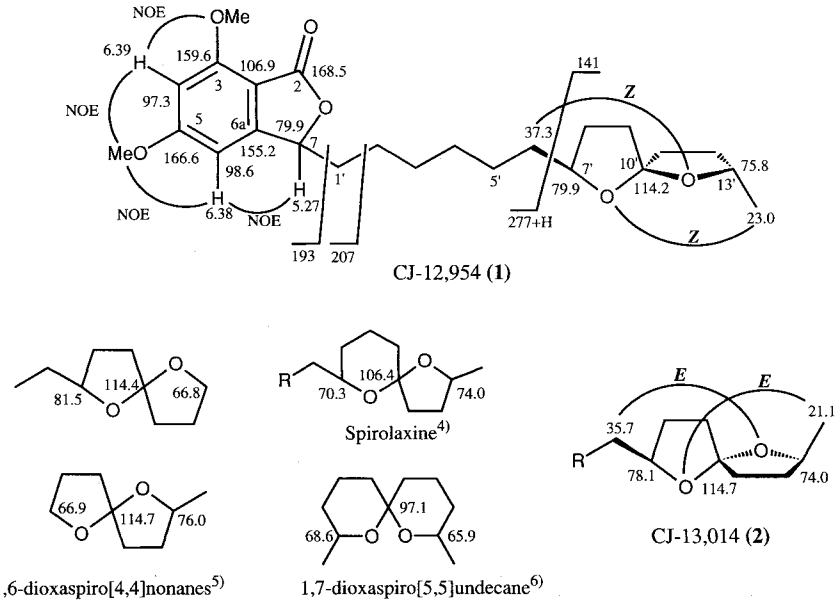
This structure was confirmed both by the comparison of spectral data with those of partially related compounds and by EI-MS analysis. Spirolaxine (**8**)[4] has a structure closely related to that of the estimated **1**. ^{1}H and ^{13}C NMR data for the phthalide and some part of extended aliphatic chain match each other, except for lack of one methoxy group in **8**. UV absorption maxima of CJ-12,954 at 290.6, 257.6 and 217.4 nm are also in good agreement with those of **8**. Comparisons of ^{13}C NMR data of the ketal carbon and two oxymethines in spiro-ketal ring

Table 1. ^{13}C NMR data (δ, ppm) of phthalides **1**~**7** (except for **5**) in $CDCl_3$.

Position	1	2	3	4	6	7
2	168.5	168.5	168.6	168.5	168.5	168.4
2a	106.9	106.9	106.7	106.9	106.9	106.8
3	159.6	159.6	159.5	159.6	159.6	159.5
-3-OMe	56.0[a]	56.0[a]	55.9	55.9	56.0[a]	55.9[a]
4	98.6[b]	98.6[b]	98.6[a]	98.6[a]	98.6[b]	98.5[b]
5	166.6	166.6	166.6	166.6	166.6	166.6
-5-OMe	55.9[a]	55.9[a]	55.9	55.9	55.9[a]	55.8[a]
6	97.3[b]	97.3[b]	97.3[a]	97.4[a]	97.4[b]	97.4[b]
6a	155.2	155.2	155.2	155.2	155.2	155.1
7	79.9	79.9	80.0	79.9	79.9	79.9
1′	34.8	34.8	34.8	34.8	34.8	34.7
2′	24.6	24.6	24.6	24.6	24.6	24.5
3′	29.4[c]	29.4[c]	29.2[b]	29.3[b]	29.3[c]	29.2[c]
4′	29.3[c]	29.3[c]	29.2[b]	29.3[b]	29.4[c]	29.3[c]
5′	25.9	25.7	29.2[b]	29.3[b]	29.5[c]	29.3[c]
6′	37.3	35.6[d]	29.2[b]	29.3[b]	29.6[c]	29.3[c]
7′	79.9	78.1	29.1[b]	29.2[b]	29.6[c]	29.4[c]
8′	30.7	30.2	23.7	25.2	29.6[c]	29.4[c]
9′	36.5[d]	35.7[d]	42.7	34.2	29.6[c]	29.4[c]
10′	114.2	114.7	209.7	73.5	29.6[c]	29.0[c]
11′	36.1[d]	35.6[d]	36.8[c]	27.9	25.7	23.7
12′	32.6	32.2	36.0[c]	39.5	39.3	43.7
13′	75.8	74.0	207.4	208.0	68.2	209.3
14′	23.0	21.1	29.9	29.9	23.4	29.7

[a~d]: Assignments marked by the same letter (a, b, c or d) are interchangeable among the same letter in the same column. Chemical shifts for 10′-OAc in **4**: 170.9 (s) and 21.1 (q). All assignments were based on those in the literatures[4~8,10] and by comparison of chemical shifts among **1**~**7** (except for **5**).

Fig. 2. Spectral data assignments (^{1}H, ^{13}C NMR and MS) of CJ-12,954 and other spiroketal compounds.



systems in the literatures[4~8] confirmed that CJ-12,954 should have 7-alkyl-2-methyl-1,6-dioxaspiro[4,4]nonane system (around 75, 114 and 80 ppm), but not 1,6-dioxaspiro[4,5]decane (around 74, 106 and 70 ppm) or 1,7-dioxaspiro[5,5]undecane (around 66, 97 and 70 ppm) systems. HREI-MS at major fragments of **1** further confirmed the structure. The base peak at *m/z* 141.0903 is consistent with a 2-methyl-1,6-dioxaspiro[4,4]nonyl part ($C_8H_{13}O_2$, $\Delta -1.1$ mmu), and the second largest peak at *m/z* 193.0501 meets requirements for a dimethoxyphthalide part ($C_{10}H_9O_4$, Δ 0.1 mmu). The remaining mass unit, 84, suggested a $(CH_2)_6$ chain between these two parts. An example of mass analysis on some bark beetle-aggregation pheromones[9] also helped us to establish 7-alkyl-1,6-dioxaspiro[4,4]nonane system. Thus, the planar structure of **1** was determined.

Compound **2** is an isomer of **1** (HREI-MS *m/z* 418.2355 calcd for $C_{24}H_{34}O_6$, $\Delta +0.1$ mmu), and has the same planar structure by all spectroscopic means. Some of the differences in ^{13}C NMR between **1** and **2** are 1.9 and 1.6 ppm downfield shifts of 14'-CH_3 and 6'-CH_2 in **1** compared to those in **2**, which is understandable as diastereomeric difference between **1** and **2**. As noted in the literatures[6~8], 14'-CH_3 and 6'-CH_2 in **1** are suggested to have the *Z*-configuration to the oxygen of the next tetrahydrofuran ring, respectively. On the other hand, 14'-CH_3 and 6'-CH_2 in **2** should have *E*-configuration to the oxygen. Thus, including the relative stereochemistry of spiroketal part, structures of CJ-12,954 and CJ-13,014 are determined as **1** and **2**. Assignments of ^{1}H and ^{13}C NMR signals were assumed depending on those in the literatures[4~8,10].

Structures of Phthalide Compounds 3 to 7

According to NMR and UV spectra, all of the 5 other compounds **3** to **7** were suggested to have the same phthalide and aliphatic chain portions like that of **1**, but to lack the spiroketal structure. The differences among these derivatives are the end parts of each aliphatic chain.

CJ-13,015 (**3**) has a molecular ion at *m/z* 418.2358 (calcd for $C_{24}H_{34}O_6$, $\Delta +0.5$ mmu). ^{13}C NMR showed 2 aliphatic ketones at 209.7 and 207.4 ppm. One of them is an acetyl group with a methyl signal at 29.9 ppm. All the other signals except the phthalide part are sp^3 triplets. ^{1}H NMR spectra showed 2H triplet (2.42 ppm) and 4H multiplet (2.67 ppm) assignable to α-positions of carbonyl groups, and the acetyl group (2.17 ppm) which has to be at the end of the alkyl chain. As the 4H multiplet has no correlation to any other protons in the decoupling experiment, the end part of the alkyl chain was determined to be $-CH_2COCH_2CH_2COCH_3$, which was also supported by the sequential loss of fragments in the EI-MS data: *m/z* 375 ($M^+ - C_2H_3O$), 347 ($M^+ - C_4H_7O$), 320 ($M^+ - C_5H_7O_2 + H$) and 305 ($M^+ - C_6H_9O_2$).

CJ-13,102 (**4**) has a molecular formula $C_{26}H_{38}O_7$ (HREI-MS: *m/z* 462.2620, $\Delta +0.4$ mmu). ^{13}C NMR

Table 2. Anti-*H. pylori* MIC and MBC of CJ-12,954 (**1**).

CJ-12,954 (ng/ml)	Growth (A_{590})	Survival (%)	
0.13×10^{-6}	0.963	100.00	
4.10×10^{-6}	0.852	92.53	
0.13×10^{-3}	0.781	85.21	
4.30×10^{-3}	0.829	54.91	
0.14	0.099	5.33	
5.00	0.009	0	MIC and MBC
0.15×10^{3}	0.008	0	
5.00×10^{3}	0.005	0	

Value is an average of triplicates.

spectra indicated two carbonyl groups: one (208.0 ppm) is acetyl group at the end like **3** and the other (170.9 ppm) is acetoxy group. The position of acetoxy group was sequentially assigned to γ-position from acetyl group at the end by H-H COSY.

CJ-13,103 (**5**), $C_{26}H_{38}O_6$ from HREI-MS (*m/z* 446.2665, $\Delta-0.1$ mmu), has exactly the same spectral features as those of **3** except for the evidences of two more methylenes in the middle of the chain: 4 additional protons at 1.22 ppm (broad singlet) in the ^{1}H NMR spectra and +28 mass fragments (*m/z* 403, 375, 348 and 333) compared to the fragments of **3** in the EI-MS spectra. Thus, the structure was determined as **5**.

CJ-13,104 (**6**), $C_{24}H_{38}O_5$ (HREI-MS: *m/z* 406.2713, $\Delta-0.3$ mmu), has 2-hydroxypropyl group at the end of the chain, which was determined both by decoupling experiment of this oxymethine and by the observation of D_2O exchangeable proton at 2.20 ppm (1H, broad) in the ^{1}H NMR spectra. EI-MS fragments at *m/z* 388 (M^+-H_2O) and 362 ($M^+-C_2H_5O+H$) also supported the structure.

CJ-13,108 (**7**) has a molecular formula $C_{24}H_{36}O_5$ (HREI-MS: *m/z* 404.2557, $\Delta-0.2$ mmu) and an acetyl group at the end like **3** from the ^{1}H and ^{13}C NMR spectra. Because no other elements were left except the methylene chain, the structure was determined as **7**.

Biological Properties

The helicobactericidal activities of the phthalide compounds are shown in Fig. 1. In the antimicrobial assay using papaer disks, the most potent phthalides gave 15 mm inhibition zones at 20 ng/disk. Noteworthy is that no antimicrobial activities were observed at a concentration of 100 μg/disk when the phthalide compounds were tested against other microorganisms such as *Bacillus stearothermophilus*, *Micrococcus luteus*, *Staphylococcus aureus* and *Pasteurella haemolytica*.

Table 2 shows the effects of **1** on growth and survival of *H. pylori*, indicating that MIC and MBC are similar (5 ng/ml). This result confirmed that the phthalide compounds were bactericidal and not just bacteriostatic.

Discussion

To find *H. pylori*-specific antibacterial compounds which are expected to have less side-effects caused by disturbance of the normal gastro-intestinal microbial flora and not to induce drug resistance of non-target microorganisms, we pursued only those extracts which had anti-*H. pylori* activity, but were inactive against a panel of other Gram-positive and -negative bacteria. By this approach, we could efficiently eliminate many extracts having antibacterial activity caused by common antibiotics at the early stage of the screening program.

As a result, we found seven new phthalide compounds **1**~**7**. The helicobactericidal activities of the phthalide compounds depend strongly on their structures (Fig. 1). Among these compounds, the spiroketal-containing **1** and **2** showed the most potent activities. The stereochemistry of the spiroketal part has no effect, but ring opening of the spiroketal to a diketone **3** reduces the potency 100-fold. The monoketone **7** is again 5-fold less potent, and after reduction to a hydroxy group **6**, the activity is virtually lost.

Because of their specific anti-*H. pylori* activity, the phthalide compounds might inhibit an *H. pylori*-specific target molecule which is not essential in the other tested microorganisms. It would therefore be interesting to examine the mode of action of the phthalide compounds further. Also, the information obtained from comparison of the phthalide compounds will be of great value during programs aiming at improvement of the *in vivo* pharmacological profile of the phthalide compounds.

The new compounds are related to spirolaxine and sporotricale[4], originally discovered as plant growth inhibitors and recently also reported to have cholesterol lowering activity[11]. Spirolaxine has helicobactericidal activity similar to **1** and **2**. These compounds may belong to the class of undecaketide derivatives such as phanerosporic acid[12] and corticiolic acid[13]. There are many known antibiotics that contain a [5,4]spiroketal, but the presence of a [4,4]spiroketal is rare: some examples are an insect pheromone[14,15] and a plant constituent[16]. We looked for [5,4]spiroketal-containing-compounds, but *Phanerochaete velutina* CL6387 does not seem to produce such compounds.

Experimental

Structural Elucidation

Spectral and physico-chemical data were obtained by the following instruments: UV, JASCO Ubest-30; IR, Shimadzu IR-470; NMR, JEOL JNM-GX270 updated with an LSI-11/73 host computer, TH-5 tunable probe and version 1.6 software; and LREI- and HREI-MS, Hitachi M-80 with an M-003 data processing system. All NMR spectra were measured in $CDCl_3$ unless otherwise indicated and peak positions are expressed in parts per million (ppm) based on the internal standard of the $CHCl_3$ peak at 7.24 ppm for ^1H and 77.0 ppm for ^{13}C.

CJ-12,954 (**1**): Colorless glass; $[\alpha]_D^{24}$ +6.0° (*c* 0.07, $CHCl_3$); UV λ_{max} (MeOH) nm (ε) 217.4 (30,000), 257.6 (14,000) and 290.6 (5,000); IR γ_{max} (KBr) cm^{-1} 2930, 1754, 1606, 1489, 1457, 1433, 1333, 1214, 1155, 1105, 1051, 1027, 985, 856, 836 and 688; EI-MS *m/z* 418 (2.7% rel. int.), 403 (1.0), 400 (1.2), 385 (2.4), 374 (4.9), 363 (4.8), 361 (16.1), 320 (33.1), 318 (7.8), 307 (6.5), 303 (3.5), 279 (5.3), 278 (8.8), 207 (22.8), 194 (29.6), 193 (80.6), 177 (6.1), 165 (12.2), 141 (100), 112 (21.3), 85 (68.1), 55 (29.1) and 41 (24.5); ^1H NMR δ 6.39 (1H, br s), 6.38 (1H, br s), 5.27 (1H, dd, J=8.1, 3.7 Hz, 7-H), 4.08 (1H, m, 13′-H), 3.93 (3H, s), 3.90 (1H, m, 7′-H), 3.87 (3H, s), 2.05~1.80 (7H, m), 1.80~1.55 (4H, m), 1.55~1.20 (9H, m) and 1.27 (3H, d, J=6.2 Hz, 14′-H).

CJ-13,014 (**2**): Colorless glass; $[\alpha]_D^{24}$ +71.2° (*c* 0.11, $CHCl_3$); UV λ_{max} (MeOH) nm (ε) 217.0 (34,000), 257.8 (16,000) and 290.4 (5,600); IR γ_{max} (KBr) cm^{-1} 2930, 1753, 1607, 1489, 1457, 1432, 1333, 1214, 1156, 1100, 1050, 1027, 916, 894, 865, 835 and 690; EI-MS *m/z* 418 (3.7% rel. int.), 403 (1.2), 400 (1.7), 385 (2.4), 374 (5.1), 363 (6.2), 361 (35.2), 360 (5.7), 345 (1.1), 320 (19.7), 318 (8.6), 307 (8.6), 303 (4.2), 279 (4.4), 278 (7.9), 207 (24.0), 194 (28.0), 193 (79.9), 191 (7.1), 177 (5.9), 165 (11.4), 141 (100), 135 (7.5), 123 (4.9), 122 (6.4), 112 (24.4), 85 (54.4), 67 (7.8), 57 (10.6), 56 (15.8) and 55 (26.0); ^1H NMR (other than phthalide part) δ 4.16 (1H, m, 13′-H), 4.01 (1H, m, 7′-H), 2.15~1.90 (7H, m), 1.65 (1H, m), 1.55~1.35 (6H, m), 1.35~1.25 (6H, m) and 1.19 (3H, d, J=6.2 Hz, 14′-H).

CJ-13,015 (**3**): Colorless glass; $[\alpha]_D^{25}$ +25.6° (*c* 0.22, $CHCl_3$); UV λ_{max} (MeOH) nm (ε) 217.4 (32,000), 257.6 (15,000) and 290.4 (5,800); IR γ_{max} (KBr) cm^{-1} 2920, 2850, 1757, 1697, 1612, 1599, 1493, 1465, 1426, 1412, 1350 (sh), 1333, 1220, 1197, 1160, 1096, 1050, 1023, 835 and 689; EI-MS *m/z* 418 (10.3% rel. int.), 400 (11.8), 375 (3.8), 347 (18.2), 320 (11.4), 305 (70.0), 207 (36.8), 194 (28.7), 193 (100), 165 (13.7), 99 (19.3) and 43 (32.3); ^1H NMR (other than phthalide part) δ 2.67 (4H, m, 11′- and 12′-H), 2.42 (2H, t, J=7.5 Hz, 9′-H), 2.17 (3H, s, 14′-H), 1.95 (1H, m, 1′-H), 1.66 (1H, m, 1′-H), 1.54 (2H, m, 7′-H), 1.42 (2H, m) and 1.24 (10H, br s).

CJ-13,102 (**4**): Colorless glass; $[\alpha]_D^{25}$ +26.8° (*c* 0.81, $CHCl_3$); UV λ_{max} (MeOH) nm (ε) 217.2 (24,000), 257.4 (11,000) and 290.4 (4,000); IR γ_{max} (KBr) cm^{-1} 2925, 2850, 1754, 1732, 1606, 1489, 1460, 1430, 1356, 1334, 1239, 1217, 1157, 1102, 1052, 1027 and 835; EI-MS *m/z* 462 (5.5% rel. int.), 402 (91.5), 363 (47.9), 345 (43.2), 207 (39.0), 194 (29.7), 193 (100), 165 (13.3) and 43 (66.0); ^1H NMR (other than phthalide part) δ 4.81 (1H, m, 10′-H), 2.42 (2H, t, J=7.5 Hz, 12′-H), 2.11 (3H, s, 14′-H), 2.00 (3H, s, 10′-OAc), 2.00~1.55 (4H, m), 1.55~1.30 (4H, m) and 1.21 (12H, br s).

CJ-13,103 (**5**): Colorless glass; $[\alpha]_D^{25}$ +26.0° (*c* 0.10, $CHCl_3$); UV λ_{max} (MeOH) nm (ε) 216.8 (26,000), 257.4 (12,000) and 290.0 (4,600); IR γ_{max} (KBr) cm^{-1} 2920, 2845, 1759, 1699, 1612, 1599, 1492, 1463, 1426, 1412, 1356, 1332, 1220, 1195, 1160, 1095, 1047, 1024, 834 and 690; EI-MS *m/z* 446 (18.5% rel. int.), 428 (19.8), 403 (6.2), 375 (21.2), 348 (13.4), 333 (100), 207 (32.0), 194 (25.1), 193 (69.6), 165 (8.8), 99 (13.7), 71 (9.7), 55 (109) and 43 (23.4); ^1H NMR (other than phthalide part) δ 2.66 (4H, m, 13′- and 14′-H), 2.42 (2H, t, J=7.5 Hz, 11′-H), 2.16 (3H, s, 16′-H), 1.94 (1H, m, 1′-H), 1.64 (1H, m, 1′-H), 1.52 (2H, m, 10′-H), 1.41 (2H, m) and 1.22 (14H, br s).

CJ-13,104 (**6**): Colorless glass; $[\alpha]_D^{25}$ +36.1° (*c* 0.41, $CHCl_3$); UV λ_{max} (MeOH) nm (ε) 216.4 (27,000), 257.6 (12,000) and 289.4 (4,400); IR γ_{max} (KBr) cm^{-1} 2915, 2845, 1755, 1736, 1611, 1599, 1493, 1462, 1426, 1336, 1220, 1197, 1160, 1097, 1052, 1026, 983, 936, 835 and 690; EI-MS *m/z* 406 (1.2% rel. int.), 388 (21.2), 362 (93.1), 207 (53.9), 194 (35.6), 193 (100), 165 (13.5), 55 (16.5), 45 (39.6) and 41 (14.9); ^1H NMR (other than phthalide part) δ 3.77 (1H, m, 13′-H), 2.20 (1H, br s, 13′-OH), 1.95 (1H, m, 1′-H), 1.65 (1H, m, 1′-H), 1.50~1.35 (4H, m), 1.22 (18H, br s) and 1.16 (3H, d, J=6.2 Hz).

CJ-13,108 (**7**): Colorless glass; $[\alpha]_D^{25}$ +44.8° (*c* 0.50, $CHCl_3$); UV λ_{max} (MeOH) nm (ε) 217.4 (30,000), 257.2 (14,000) and 290.2 (5,200); IR γ_{max} (KBr) cm^{-1} 2915, 2845, 1756, 1707, 1611, 1600, 1492, 1464, 1425, 1355, 1333, 1219, 1195, 1159, 1099, 1050, 1023, 995, 974, 833 and 690; EI-MS *m/z* 404 (58.7% rel. int.), 347 (81.5), 207 (43.8), 194 (31.1), 193 (100), 165 (13.4), 58 (15.5) and 43 (42.8); ^1H NMR (other than phthalide part) δ 2.36 (2H, t, J=7.3 Hz, 12′-H), 2.07 (3H, s, 14′-H), 1.91 (1H, m, 1′-H), 1.64 (1H, m, 1′-H), 1.50 (2H, m, 11′-H), 1.38 (2H, m) and 1.18 (16H, br s).

Antimicrobial Activity

H. pylori 41, *Bacillus stearothermophilus* ss calidolactis C593, *Micrococcus luteus* ATCC9341, *Pasteurella heamolytica* 59B018 and *Staphylococcus aureus* 01-A-005 were obtained from our culture collection. Stock cultures of *H. pylori* 41 were stored at −80°C in brucella broth (BBL Microbiology Systems) supplemented with 2.5% heat-inactivated bovine serum and 15% glycerol. *H. pylori* 41 was grown on brucella agar (BBL Microbiology Systems) supplemented with 10% FBS. The other microorganisms were grown on Medium 11 (Difco). Antimicrobial activity was tested using paper disks (i.d. 8 mm, Advantec), and activity was observed after 24-hour incubation at 37°C (55°C for *B. stearothermophilus*).

For determination of MBC and MIC, *H. pylori* was grown in brucella broth supplemented with 2.5% FBS using shallow petridishes[17]. Growth was monitored by measuring the absorption at 590 nm, and viability was determined by cfu count of plated 10-fold serial dilutions[17].

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