Antitubercular Constituents from the Stem Wood of *Cinnamomum kotoense*

Fu-Chi Chen,[†] Chien-Fang Peng,[‡] Ian-Lih Tsai,[†] and Ih-Sheng Chen*,[†]

Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan 807, Republic of China, and Faculty of Biomedical Laboratory Sciences, Kaohsiung Medical University, Kaohsiung, Taiwan 807, Republic of China

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Five new compounds, kotolactone A (**1**), kotolactone B (**2**), secokotomolide (**3**), kotodiol (**4**), and 2-acetyl-5-dodecylfuran (**5**), and 36 known compounds have been isolated from the stem wood of *Cinnamomum kotoense*. The structures of these new compounds were determined by means of spectroscopic analysis. The known butanolides, isoobtusilactone A (**6**) and lincomolide B (**7**), showed in vitro antitubercular activities with MIC values of 22.48 and 10.16 μM, respectively, against *Mycobacterium tuberculosis* 90-221387.

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*. For over thirty years, no antitubercular agents with new mechanisms of action have been developed, and the worldwide incidence of multidrug-resistant *M. tuberculosis* has become an increasingly serious public health problem. Searching for structurally new types of drugs effective against this infectious disease has therefore become vitally important. Recently, about 200 species of Formosan plants have been screened for antitubercular activity, and *Cinnamomum kotoense* Kanehira et Sasaki (Lauraceae) was shown to be one of the active species. *C. kotoense* is a small evergreen tree, endemic to Lanyu Island of Taiwan,[1] and recently has been cultivated as an ornamental plant. The chemical constituents and the biological activity of this plant have not yet been investigated, but examination of the stem wood has led to the isolation of five new compounds (**1**−**5**). This paper reports the structural elucidation of these new compounds and the antitubercular activity of the isolates.

Results and Discussion

Kotolactone A (**1**) was isolated as colorless needles. The EIMS afforded the molecular ion $[M]^+$ at *m/z* 520, implying a molecular formula of $C_{33}H_{60}O_4$, which was subsequently confirmed by the HREIMS. UV absorption at 243 nm and IR data (1770 and 1678 cm^{-1}) indicated the presence of an α-alkylidene-substituted γ-lactone skeleton, and the ^1H and ^{13}C NMR spectra (δ 168.1, C-5; δ 126.2, C-6) suggested the presence of a butyrolactone moiety[2] connected to another aliphatic chain by an acetal linkage.[3] The ^1H NMR spectrum of **1** (Table 1) showed a high degree of similarity to that of machilactone (**42**),[3] indicating *E*-geometry (δ 7.09, H-1′) of the double bond and *cis*-configurations of CH_3-3a (δ 1.71)/H-6a (δ 4.89) and H-6a (δ 4.89)/H_2-1″ (δ 1.68). However, both side chains at C-1′ and C-1″ in **1** (*m/z* 520) contain four methylene groups less than those in machilactone (**42**, *m/z* 632). A significant fragment at *m/z* 337 in the EIMS spectrum of **1** suggested the presence of a tetradecylidene side chain at C-6. On the basis of the data above, the structure of kotolactone A was elucidated as **1**, which was further confirmed by COSY, DEPT, HETCOR, NOESY (Figure 1), and HMBC (Figure 2) experiments.

Kotolactone B (**2**) was isolated as colorless needles. Its molecular formula was established as $C_{33}H_{60}O_4$ by EIMS ($[M]^+$, *m/z* 520) and HREIMS. UV absorption at 243 nm and IR data (1771 and 1680 cm^{-1}) were similar to those of **1**. The ^1H NMR spectrum (Table 1) of **2** was similar to that of **1**, except that H-2 resonated at δ 5.27 and H-1″ at δ 1.63 in **2**, instead of δ 5.00 and 1.68, respectively, suggesting that **2** is a stereoisomer of **1**. H-1′ resonating at δ 7.09 suggests that **2** possesses *E*-geometry. A *cis*-relationship between CH_3-3a (δ 1.64) and H-6a (δ 4.79) and between H-6a (δ 4.79) and H-2 (δ 5.27) were supported by NOESY correlations (Figure 1). A significant fragment at *m/z* 337 also appeared in the EIMS of **2**, suggesting the presence of a tetradecylidene side chain at C-6. According to the above data, the structure of kotolactone B was elucidated as **2**, which was confirmed by COSY, DEPT, HETCOR, NOESY (Figure 1), and HMBC (Figure 2) experiments.

Secokotomolide (**3**) was obtained as a colorless oil. Its molecular formula of $C_{17}H_{30}O_4$ was determined by EIMS ($[M]^+$, *m/z* 298) and HREIMS. UV absorption at 220 nm and IR absorption bands are due to the presence of hydroxyl (3447 cm^{-1}), ester (1725 cm^{-1}), and carbonyl (1643 cm^{-1}) groups, suggesting that **3** is a secobutanolide.[2] The ^1H NMR spectrum of **3** was similar to that of secolincomolide A,[4] except that **3** had one methylene unit less in the alkyl side chain. The negative optical rotation $\{[\alpha]^{25}_D$ −11.5°} of **3** indicated that C-3 possesses an *R* configuration, compared with that of secolincomolide A $\{[\alpha]^{25}_D$ −11.3°}.[4] Thus, the structure of secokotomolide was elucidated as **3**, which was further confirmed by COSY, DEPT, HETCOR, and NOESY (Figure 1) experiments.

Kotodiol (**4**) was isolated as colorless needles. Its molecular formula of $C_{28}H_{58}O_2$ was determined by EIMS ($[M]^+$, *m/z* 426) and HREIMS. The IR spectrum showed a hydroxyl absorption at 3416 cm^{-1}. The ^1H NMR spectrum showed an oxymethine proton at δ 4.83 (1H, t, J = 5.4 Hz) that suggested the presence of a dihydroxylated carbon resonating at δ 101.7 in the ^{13}C NMR spectrum. A residual *n*-heptacosyl group [δ 0.88 (3H, t, J = 7.2 Hz, H-28), 1.25 (46H, s, H-4-H-26), 1.27 (2H, m, H-3), 1.39 (2H, m, H-27), 1.66 (2H, m, H-2)] was observed in the ^1H NMR spectrum. According to the above data, the structure of kotodiol was elucidated as **4**, which was further confirmed by COSY, ^{13}C NMR, HMQC, and HMBC (Figure 2) experiments.

2-Acetyl-5-dodecylfuran (**5**) was obtained as colorless needles. Its molecular formula of $C_{18}H_{30}O_2$ was determined by EIMS ($[M]^+$, *m/z* 278) and HREIMS. UV absorptions at 211 and 275 nm indicated the presence of a 2-acetylfuran moiety.[6] The IR spectrum showed an absorption band for

* To whom correspondence should be addressed. Tel: +886-7-3121101, ext. 2191. Fax: +886-7-3210683. E-mail: m635013@kmu.edu.tw.
[†] Graduate Institute of Natural Products.
[‡] Faculty of Biomedical Laboratory Sciences.

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Table 1. ^{1}H NMR Data of Machilactone (**42**), Kotolactone A (**1**), and Kotolactone B (**2**) in $CDCl_3$ (J Hz)

position	**42** (600 MHz)	**1** (400 MHz)	**2** (400 MHz)
2	5.01 (1H, t, J = 4.8)	5.00 (1H, t, J = 4.8)	5.27 (1H, t, J = 5.2)
6a	4.89 (1H, d, J = 1.2)	4.89 (1H, d, J = 1.6 Hz)	4.79 (1H, d, J = 1.6)
1′	7.09 (1H, td, J = 7.8, 1.8)	7.09 (1H, td, J = 7.8, 1.8)	7.02 (1H, td, J = 8.0, 1.2)
2′	2.40 (2H, m)	2.41 (2H, m)	2.38 (2H, m)
3′	1.50 (2H, m)	1.51 (2H, m)	1.51 (2H, m)
4′−17′	1.26 (28H, s)		
4′−13′		1.25 (20H, brs)	1.25 (20H, brs)
1″	1.68 (2H, m)	1.68 (2H, m)	1.63 (2H, m)
2″	1.37 (2H, m)	1.38 (2H, m)	1.32 (2H, m)
3″−14″	1.26 (28H, s)		
3″−12″		1.25 (20H, brs)	1.25 (20H, brs)
CH_3-3a	1.71 (3H, s)	1.71 (3H, s)	1.64 (3H, s)
18′	0.88 (3H, t, J = 7.2)		
17″	0.88 (3H, t, J = 7.2)		
14′		0.87 (3H, t, J = 6.8)	0.87 (3H, t, J = 6.4)
13″		0.87 (3H, t, J = 6.8)	0.87 (3H, t, J = 6.4)

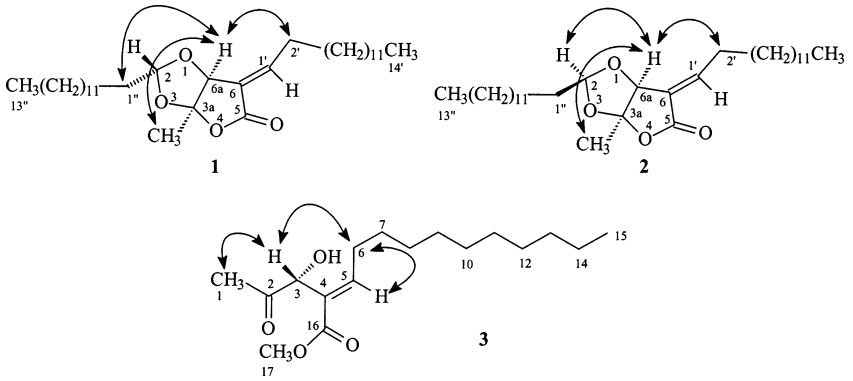


Figure 1. NOESY correlations of **1**, **2**, and **3**.

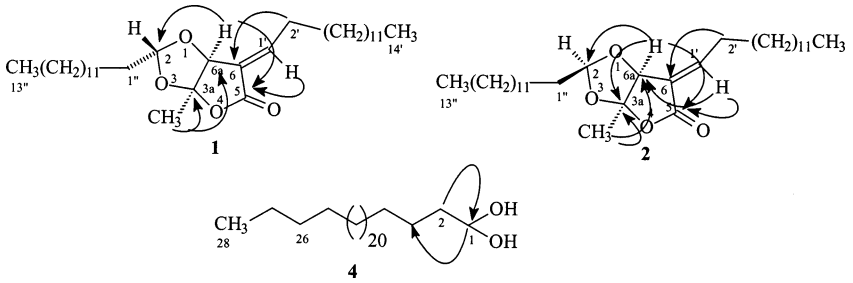


Figure 2. HMBC connectivities of **1**, **2**, and **4**.

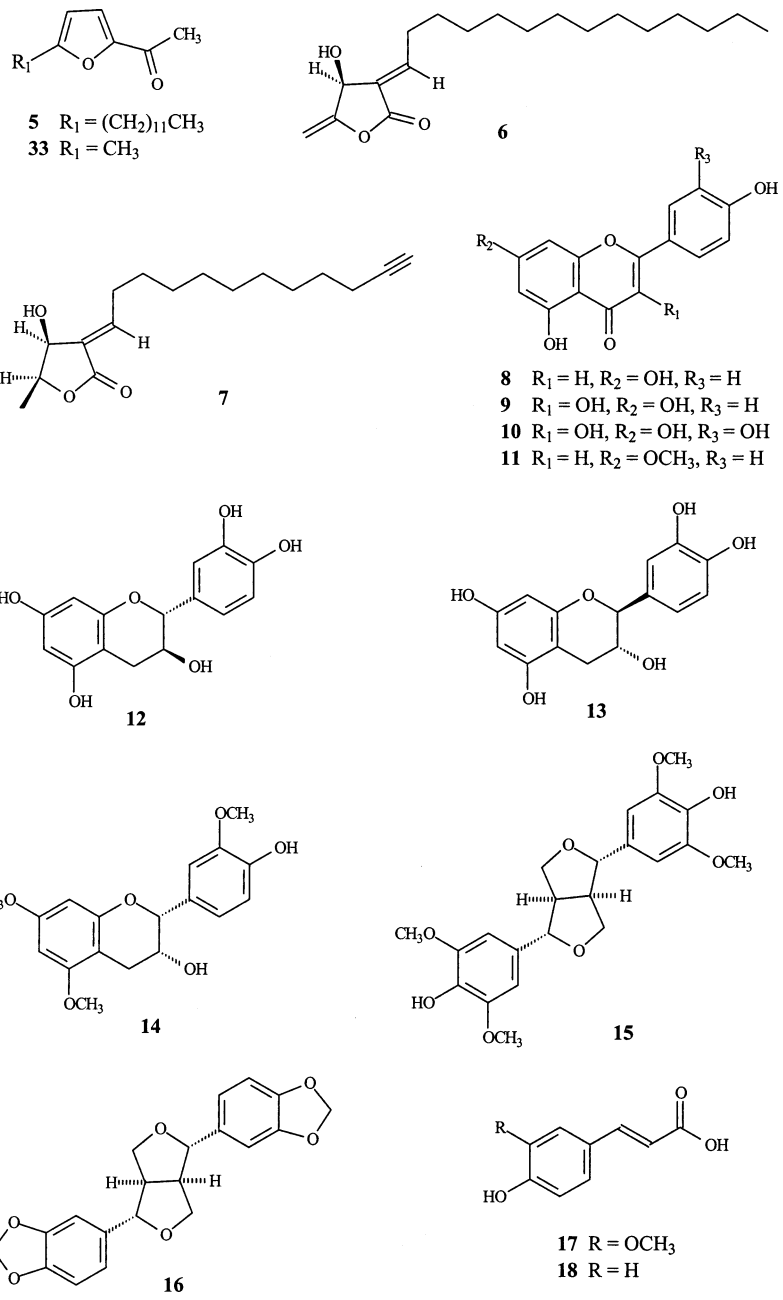
a conjugated ketone group at 1676 cm^{-1}. The ^{1}H NMR spectrum of **5** showed an acetyl group at δ 2.43 (3H, s), a pair of olefinic protons at δ 6.15, 7.10 (each 1H, d, J = 3.2 Hz), and a dodecyl group [δ 0.87 (3H, t, J = 6.8 Hz, H-17), 1.24 (18H, brs, H-8-H-16), 1.67 (2H, m, H-7), 2.69 (2H, t, J = 7.6 Hz, H-6)]. According to these observations, the structure of **5** was elucidated to be 2-acetyl-5-dodecylfuran, which was further supported by prominent fragment ions such as *m/z* 109, 123, 137, 179, 193, and 235 in the EIMS. 2-Acetyl-5-dodecylfuran (**5**) was first isolated from nature, though it has been previously synthesized by Scheinkönig et al.[7]

The known compounds isoobtusilactone A (**6**),[8] lincomolide B (**7**),[9] apigenin (**8**),[10] kaempferol (**9**),[11] quercetin (**10**),[12] genkwanin (**11**),[10] (+)-catechin (**12**),[13] (−)-catechin (**13**),[14] (−)-4′-hydroxy-5,7,3′-trimethoxyflavan-3-ol (**14**),[15] (±)-syringaresinol (**15**),[16] (−)-sesamin (**16**),[17] *trans*-ferulic acid (**17**),[18] *trans*-coumaric acid (**18**),[19] 2,6-dimethoxy-1,4-benzoquinone (**19**),[20] syringaldehyde (**20**),[21] vanillin (**21**),[22] 4-hydroxybenzaldehyde (**22**),[23] protocatechuic acid (**23**),[24] benzoic acid (**24**),[25] a mixture of sitostenone (**25**)[26] and stigmasta-4,22-dien-3-one (**26**),[27] a mixture of β-sitosterol (**27**) and stigmasterol (**28**),[28] a mixture of β-sitosteryl-3-*O*-β-D-glucoside (**29**)[28,29] and stigmasteryl-3-*O*-β-D-glucoside (**30**),[28] squalene (**31**),[30] *trans*-phytol (**32**),[30] 2-acetyl-5-methylfuran (**33**),[31] a mixture of methyl palmitate (**34**) and methyl stearate (**35**),[32] lauric acid (**36**),[33] a mixture of palmitic acid (**37**),[34] margaric acid (**38**),[35] and stearic acid (**39**),[36] docosanoic acid (**40**),[37] and tetracosane (**41**)[38] were identified by comparisons of physical and spectroscopic data ([α]$_D$, UV, IR, ^{1}H NMR, and MS] with authentic samples or literature data.Bioactivity-guided fractionation of the active $CHCl_3$ fraction led to the isolation of isoobtusilactone A (**6**) and lincomolide B (**7**) as active constituents with antitubercular activity, showing MIC values of 22.48 and 10.16 μM, respectively. Vanillin (**21**) and lauric acid (**36**) showed MIC values of 82.24 and 62.5 μM, respectively. Rifampin (MIC of 0.24 μM) was used as the positive control.

Experimental Section

General Experimental Procedures. All melting points were determined on a Yanaco micro-melting point apparatus and are uncorrected. IR spectra (KBr or neat) were taken on a Perkin-Elmer System 2000 FT-IR spectrometer. UV spectra were obtained on a Jasco UV-240 spectrophotometer in EtOH. EIMS spectra were recorded on a Micromass TRIO-2000

Chart 1

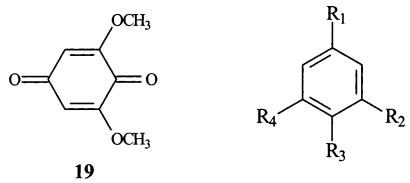


spectrometer. HRMS spectra were recorded on a Finnigan/Thermo Quest NAT mass spectrometer. 1D and 2D NMR spectra were recorded on a Varian Unity 400 and Bruker AV-500 spectrometer. Chemical shifts are given in ppm (δ), with TMS as the internal standard. Optical rotations were measured on a Jasco P-1020 polarimeter. Silica gel (70-230, 230−400 mesh) (Merck) was used for CC, and silica gel 60 F-254 (Merck) was used for TLC and preparative TLC.

Plant Material. The stem wood of *C. kotoense* was purchased from Wandan, Pingtung County, Taiwan, in September 2002 and identified by one of the authors (I.S.C.). A voucher specimen was deposited in the herbarium of the School of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan, Republic of China.

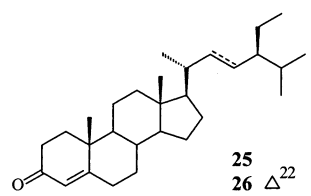
Extraction and Isolation. The dried stem wood (11.5 kg) of *C. kotoense* was extracted with cold MeOH to yield the MeOH extract (1.16 kg). The extract was partitioned between H_2O and $CHCl_3$ (1:1) to afford a $CHCl_3$-soluble fraction (fraction A, 410 g). The H_2O extract was partitioned with *n*-BuOH (1:1) and afforded an *n*-BuOH-soluble fraction (fraction B, 90 g). Part of fraction A (100 g) was chromatographed on a Si gel (2.5 kg) column with a step gradient of *n*-hexane (100%), *n*-hexane−EtOAc (99:1, 97:3, 95:5, 9:1, 4:1, 7:3, 3:2, 1:1, 3:7), EtOAc (100%), EtOAc−MeOH (9:1, 4:1, 1:1), and MeOH (100%) to give 15 fractions (1−15). Fraction A5 (1.091 g) was chromatographed on Si gel (30 g) eluting with *n*-hexane, gradually increasing the polarity with EtOAc to give 20 fractions (each 250 mL, A5-1−A5-20). Fraction A5-4 (182 mg) was further purified by preparative TLC (*n*-hexane−EtOAc, 40:1) to afford **36** (145 mg). Fraction A5-6 (172 mg) was further purified by preparative TLC (*n*-hexane−EtOAc, 20:1) to obtain **40** (132 mg). Fraction A5-9 (105 mg) was further purified by preparative TLC (CH_2Cl_2−MeOH, 20:1) to obtain **41** (96 mg). Fraction A6 (1.224 g) was chromatographed on Si gel (30 g) eluting with *n*-hexane, gradually increasing the polarity with EtOAc to give 15 fractions (each 250 mL, A6-1−A6-15). Fraction A6-7 (89 mg) was further purified by preparative TLC (*n*-hexane−$CHCl_3$, 1:1) to obtain **4** (23 mg). Fraction A6-12 (112 mg) was purified by preparative TLC (*n*-hexane−EtOAc, 5:1) to obtain a mixture of **27** and **28** (39 mg). Fraction A6-14 (88 mg) was purified by preparative TLC (*n*-hexane−EtOAc, 4:1) to yield a mixture of **25** and **26** (26 mg). Fraction A7 (0.02 g)

Chart 2

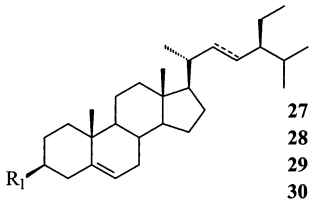


19

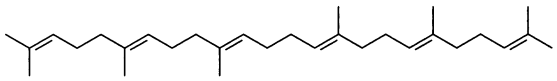
20 R_1 = CHO, R_2 = OCH_3, R_3 = OH, R_4 = OCH_3
21 R_1 = CHO, R_2 = OCH_3, R_3 = OH, R_4 = H
22 R_1 = CHO, R_2 = H, R_3 = OH, R_4 = H
23 R_1 = COOH, R_2 = OH, R_3 = OH, R_4 = H
24 R_1 = COOH, R_2 = H, R_3 = H, R_4 = H



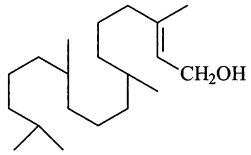
25
26 Δ^{22}



27 R_1 = OH
28 R_1 = OH, Δ^{22}
29 R_1 = GluO
30 R_1 = GluO, Δ^{22}



31

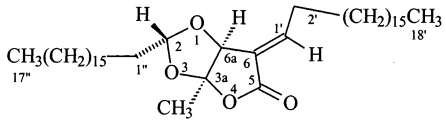


32

$CH_3(CH_2)_nCOOCH_3$
34 n = 14
35 n = 16

$CH_3(CH_2)_nCOOH$
36 n = 10
37 n = 14
38 n = 15
39 n = 16
40 n = 20

$CH_3(CH_2)_{22}CH_3$
41



42

was purified by preparative TLC (*n*-hexane−Me_2CO, 5:1) to obtain **3** (3 mg). Fraction A-8 (9.929 g) was recrystallized with MeOH to obtain **14** (43 mg). The mother liquor (9.873 mg) was chromatographed on Si gel (300 g) eluting with a gradient of *n*-hexane, $CHCl_3$, EtOAc, and MeOH to give 10 fractions (each 250 mL, A8-1−A8-10). Fraction A8-3 (2.699 mg) was chromatographed on Si gel (90 g) eluting with a gradient of *n*-hexane, EtOAc, and MeOH to give 10 fractions (each 250 mL, A8-3-1−A8-3-10). Fraction A-8-3-3 (1.202 g) was chromatographed on Si gel (30 g) eluting with a gradient of $CHCl_3$, EtOAc, Me_2CO, and MeOH to give six fractions (each 250 mL, A8-3-3-1−A8-3-3-6). Fraction A8-3-3-4 was further purified by preparative TLC (*n*-hexane−EtOAc, 20:1) to yield a mixture of **37**, **38**, and **39** (112 mg). Fraction A8-3-5 (0.775 g) was resubjected to Si gel (30 g) eluting with a gradient of *n*-hexane, CH_2Cl_2, and EtOAc to give 10 fractions (each 250 mL, A8-3-5-1−A8-3-5-10). Fraction A-8-3-5-2 (98 mg) was purified by preparative TLC (*n*-hexane−EtOAc, 40:1) to obtain a mixture of **34** and **35** (70 mg). Fraction A8-7 (0.523 g) was subjected to Si gel (20 g) eluting with a gradient of *n*-hexane and EtOAc to give 10 fractions (each 250 mL, A8-7-1−A8-7-10). Fraction A8-7-1 was purified by preparative TLC (*n*-hexane−Me_2CO, 100:1) to afford **31** (1.95 mg). Fraction A8-7-2 (12 mg) was further purified by preparative TLC (*n*-hexane−Me_2CO, 20:1) to obtain **32** (1.5 mg). Fraction A9 (5.400 g) was chromatographed on Si gel (150 g) eluting with a gradient of *n*-hexane (100%), *n*-hexane−EtOAc (95:5, 9:1, 4:1, 1:1), EtOAc (100%), EtOAc−MeOH (9:1, 4:1, 1:1), and MeOH (100%) to give 15 fractions (each 250 mL, A9-1−A9-15). Fraction A9-2 (0.675 g) was resubjected to Si gel (20 g) eluting with a gradient of *n*-hexane (100%), *n*-hexane−EtOAc (95:5, 4:1, 3:2, 1:1), and EtOAc (100%) to yield nine fractions (each 250 mL, A9-2-1−A9-2-9). Fraction A9-2-2 (123 mg) was purified by preparative TLC (*n*-hexane−EtOAc, 20:1) to obtain **1** (88 mg). Fraction A9-2-5 (93 mg) was purified by preparative TLC (*n*-hexane−EtOAc, 20:1) to afford **2** (42 mg). Fraction A10 (1.59 g) was chromatographed on Si gel (45 g) eluting with $CHCl_3$−MeOH step gradients to give 15 fractions (each 250 mL, A10-1−A10-15). Fraction A10-7 (122 mg) was purified by preparative TLC (CH_2Cl_2−MeOH, 20:1) to yield **24** (18 mg). Fraction A10-9 (105 mg) was purified by preparative TLC ($CHCl_3$−Me_2CO, 10:1) to obtain **33** (30 mg). Fraction A10-10 (62 mg) was recrystallized by MeOH to obtain **20** (17 mg). Fraction A11 (0.991 g) was chromatographed on Si gel (30 g) eluting with *n*-hexane, $CHCl_3$, EtOAc, and MeOH to give 16 fractions (each 250 mL, A11-1−A11-16). Fraction A11-10 (43 mg) was purified by preparative TLC ($CHCl_3$−Me_2CO, 20:1) to afford **17** (9 mg). Fraction A11-12 (38 mg) was purified by preparative TLC ($CHCl_3$−Me_2CO, 20:1) to obtain **18** (12 mg). Fraction A11-13 (105 mg) was purified by preparative TLC (*n*-hexane−Me_2CO, 20:1) to obtain **7** (23 mg). Fraction A11-14 (16 mg) was purified by preparative TLC (*n*-hexane−Me_2CO, 20:1) to yield **6** (3.3 mg). Fraction A12 (2.762 g) was subjected to Si gel (80 g) eluting with $CHCl_3$−MeOH step gradients to give 16 fractions (each 250 mL, A12-1−A12-16). Fraction A12-1 (92 mg) was purified by preparative TLC (*n*-hexane−Me_2CO, 1:1) to yield **15** (19 mg). Fraction A-12-2 (36.0 mg) was purified by preparative TLC (*n*-hexane−Me_2CO, 10:1) to obtain **19** (13.1 mg). Fraction A-12-10 (52 mg) was recrystallized from MeOH to obtain **16** (13 mg), mp 119−121 °C (lit.[17] mp 119−121 °C). Fraction A12-11 (103 mg) was purified by preparative TLC (EtOAc−MeOH, 10:1) to obtain **23** (20 mg). Fraction A12-13 (86 mg) was purified by preparative TLC ($CHCl_3$−Me_2CO, 1:1) to yield **8** (38 mg). The Me_2CO-soluble part (1.436 g) of fraction A13 (2.056 g) was chromatographed on Si gel (45 g) eluting with $CHCl_3$−Me_2CO−MeOH step gradients to give 11 fractions (each 250 mL, A13-1−A13-11). Fraction A13-1 (10 mg) was purified by preparative TLC (*n*-hexane−EtOAc, 4:1) to obtain **5** (1.2 mg). Fraction A13-5 (123 mg) was recrystallized with MeOH to yield **21** (43 mg). Fraction A13-7 (89 mg) was purified

Table 2. ^{13}C NMR Data of Machilactone (**42**), Kotolactone A (**1**), and Kotolactone B (**2**) in $CDCl_3$

position	**42** (150 MHz)	**1** (100 MHz)	**2** (100 MHz)
2	103.9	103.9	108.2
3a	109.8	109.8	109.9
5	168.1	168.1	167.3
6	126.3	126.2	127.6
6a	78.4	78.4	78.7
1′	150.8	150.9	149.0
2′	30.2	30.1	30.2
3′	28.2	28.1	28.2
4′−15′ & 3″−14″	23.6, 26.9, 27.2, 29.3, 29.4, 29.5, 29.6, 29.7		
4′−11′ & 3″−10″		23.6, 29.2, 29.3, 29.4, 29.5	23.6, 29.2, 29.3, 29.4, 29.5, 29.7
1″	32.7	32.6	35.0
2″	31.9	31.9	31.9
CH_3-3a	22.7	22.6	22.5
12′,11″	32.0	31.9	31.9
13′,12″	22.4	22.6	22.7
14′,13″		14.1	14.1
18′,17″	14.1		

Table 3. MIC Values of Test Compounds from Stem Wood of *C. kotoense* against *M. tuberculosis* 90-221387

compound	MIC[a]
kotolactone A (**1**)	40.07 μM
kotodiol (**4**)	117.37 μM
isoobtusilactone A (**6**)	22.48 μM
lincomolide B (**7**)	10.16 μM
(−)-4′-hydroxy-5,7,3′-trimethoxyflavan-3-ol (**14**)	150.60 μM
(±)-syringaresinol (**15**)	239.29 μM
syringaldehyde (**20**)	137.26 μM
vanillin (**21**)	82.24 μM
4-hydroxybenzaldehyde (**22**)	368.85 μM
protocatechuic acid (**23**)	649.35 μM
benzoic acid (**24**)	327.86 μM
mixture of β-sitosterol (**27**) and stigmasterol (**28**)	64 μg/mL
mixture of β-sitosteryl-3-*O*-β-D-glucoside (**29**) and stigmasteryl-3-*O*-β-D-glucoside (**30**)	50 μg/mL
2-acetyl-5-methylfuran (**33**)	322.58 μM
mixture of methyl palmitate (**34**) and methyl stearate (**35**)	45 μg/mL
lauric acid (**36**)	62.50 μM
mixture of palmitic acid (**37**), margaric acid (**38**), and stearic acid (**39**)	25 μg/mL
rifampin (positive control)	0.24 μM

[a] Mixture shown as μg/mL.

by preparative TLC ($CHCl_3$−MeOH, 10:1) to obtain **22** (13 mg). Fraction A13-9 (77 mg) was recrystallized with MeOH to afford a mixture of **29** and **30** (42 mg). Fraction A14 (2.180 g) was chromatographed on Si gel (70 g) eluting with $CHCl_3$−MeOH step gradients to give 20 fractions (each 250 mL, A14-1−A14-20). Fraction A14-12 (36 mg) was purified by preparative TLC (CH_2Cl_2−MeOH, 8:1) to obtain **11** (4.2 mg). Part of fraction B (10 g) was subjected to Si gel (300 g) eluting with $CHCl_3$−MeOH (20:1, 10:1, 5:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:5, MeOH) to give 20 fractions (B1−B20). Fraction B16 (22 mg) was purified by preparative RP-18 TLC (CH_3CN−H_2O, 2:1) to yield **12** (2.2 mg). Fraction B17 (25 mg) was purified by preparative RP-18 TLC (MeOH−H_2O, 3:1) to yield **10** (4.4 mg). Fraction B-18 (24 mg) was purified by preparative RP-18 TLC (CH_3CN−H_2O, 1:1) to yield **13** (1.2 mg). Fraction B-19 (25 mg) was purified by preparative RP-18 TLC (MeOH−H_2O, 2:1) to yield **9** (4.8 mg).

Kotolactone A (1): colorless needles (MeOH); mp 56−58 °C; $[\alpha]^{25}_D$ +23.95° (*c* 2.5, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 243 (4.79) nm; IR (KBr) ν_{max} 1770, 1678 (α,β-unsaturated-γ-lactone) cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 520 ([M]$^+$, 14), 477 (23), 369 (20), 337 (11), 309 (10), 291 (60), 139 (12), 123 (10), 111 (16), 97 (15), 95 (15), 81 (14), 71 (10), 69 (25), 67 (16), 57 (39), 55 (40), 43 (100), 41 (30); HREIMS *m/z* 520.4546 (calcd for $C_{33}H_{60}O_4$, 520.4539).

Kotolactone B (2): colorless needles (MeOH); mp 55−57 °C; $[\alpha]^{25}_D$ +20.95° (*c* 2.2, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 242 (4.79) nm; IR (KBr) ν_{max} 1771, 1680 (α,β-unsaturated-γ-lactone) cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m/z* 520 ([M]$^+$, 2), 477 (3), 337 (3), 309 (8), 292 (20), 291 (100), 273 (20), 263 (12), 245 (22), 221 (16), 193 (15), 179 (14), 165 (11), 135 (13), 123 (12), 95 (16), 81 (14), 67 (12); HREIMS *m/z* 520.4488 (calcd for $C_{33}H_{60}O_4$, 520.4491).

Secokotomolide (3): colorless oil; $[\alpha]^{25}_D$ −11.5° (*c* 0.28, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 220 (4.44) nm; IR (KBr) ν_{max} 3447 (OH), 1725 (ester), 1643 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.87 (3H, t, J = 6.8 Hz, CH_3-15), 1.25 (14H, brs, H-8−H-14), 1.51 (2H, m, H-7), 2.15 (3H, s, $COCH_3$), 2.34 (2H, q, J = 7.4 Hz, H-6), 3.72 (3H, s, OCH_3), 3.89 (1H, brs, OH-3), 4.89 (1H, brs, H-3), 7.07 (1H, t, J = 7.6 Hz, H-5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1 (C-15), 22.7 (C-14), 24.3 (C-1), 28.65 (C-6), 28.69 (C-7), 29.3, 29.4, 29.6 (C-8−12), 31.9 (C-13), 52.0 (OCH_3), 73.3 (C-3), 129.6 (C-4), 149.2 (C-5), 166.5 (C-16), 206.4 (C-2); EIMS *m/z* 298 ([M]$^+$, 24), 297 (98), 265 (100), 261 (11), 247 (19), 237 (18), 219 (53), 192 (12), 191 (15), 177 (17), 163 (26), 153 (48), 149 (41), 135 (70), 121 (50), 109 (45), 95 (47), 81 (48), 67 (27), 55 (24); HREIMS: 298.2135 (calcd for $C_{17}H_{30}O_4$, 298.2144).

Kotodiol (4): colorless needles (Me_2CO); mp 59−61°C; IR (KBr) ν_{max} 3416 (OH) cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 0.88 (3H, t, J = 7.2 Hz, H-28), 1.25 (46H, s, H-4-H-26), 1.27 (2H, m, H-3), 1.39 (2H, m, H-27), 1.66 (2H, m, H-2), 4.83 (1H, t, J = 5.4 Hz, H-1); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 14.1 (C-28), 22.1 (C-3), 22.7 (C-27), 29.2−29.7 (C-4-C-25), 31.9 (C-26), 34.4 (C-2), 101.7 (C-1); EIMS *m/z* 426 ([M]$^+$, 5), 424 (19), 214 (14), 213 (100), 125 (13), 111 (22), 97 (26), 83 (21), 67 (22); HREIMS *m/z* 426.4362 (calcd for $C_{28}H_{58}O_2$, 426.4359).

2-Acetyl-5-dodecylfuran (5): colorless needles (Me_2CO); mp 148−150 °C; UV (MeOH) λ_{max} (log ϵ) 211 (4.31), 275 (3.96) nm; IR (KBr) ν_{max} 1676 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.87 (3H, t, J = 6.8 Hz, H-17), 1.24 (18H, brs, H-8-H-16), 1.67 (2H, m, H-7), 2.43 (3H, s, CH_3CO), 2.69 (2H, t, J = 7.6 Hz, H-6), 6.15 (1H, d, J = 3.2 Hz, H-4), 7.10 (1H, d, J = 3.2 Hz, H-3); EIMS *m/z* 278 ([M]$^+$, 57), 263 (3), 235 (35), 217 (21), 209 (40), 193 (31), 179 (27), 161 (31), 147 (26), 137 (100), 124 (48), 123 (58), 111 (37), 109 (19), 95 (54), 81 (43), 67 (21); HREIMS *m/z* 278.2246 (calcd for $C_{18}H_{30}O_2$, 278.2251).

Antitubercular Activity Assay. Antitubercular activity of each test compound was evaluated using *Mycobacterium tuberculosis* 90-221387. Middlebrook 7H10 agar was used to determine the MICs as recommended by the proportion method.[39] Briefly, each test compound was added to Middlebrook 7H10 agar supplemented with OADC (oleic acid-albumin-dextrose-catalase) at 50−56 °C by a serial dilution to yield a final concentration of 100 to 0.8 μg/mL. Ten milliliters of each concentration of test compound-containing medium was dispensed into plastic quadrant Petri dishes. Several colonies of test isolate of *M. tuberculosis* were selected to make the suspension with Middlebrook 7H9 broth and used as the initial inoculum. The inoculum of test isolate of *M. tuberculosis* was prepared by diluting the initial inoculum in Middlebrook 7H9 broth until the turbidity was reduced to that equivalent to the McFarland no. 1 standard. Final suspensions were prepared by adding Middlebrook 7H9 broth and preparing 10^{-2} dilutions of the standardized bacterial suspensions. After solidification of the Middlebrook 7H10 medium, 33 μL of the 10^{-2} dilution of the standardized bacterial suspensions was placed on each quadrant of the agar plates. The agar plates were then incubated at 35 °C with 10% CO_2 for 2 weeks. The minimal inhibitory concentration (MIC) is the lowest concentration of test compounds that completely inhibits the growth of the test isolate of *M. tuberculosis*, as detected by the unaided eye.

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