Constituents of the Root Wood of *Zanthoxylum wutaiense* with Antitubercular Activity

Hung-Yi Huang,[†] Tsutomu Ishikawa,[‡] 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, Faculty of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi, Inage, Chiba 263-8522, Japan, and Faculty of Biomedical Laboratory Science, College of Health Sciences, Kaohsiung Medical University, Kaohsiung, Taiwan 807, Republic of China

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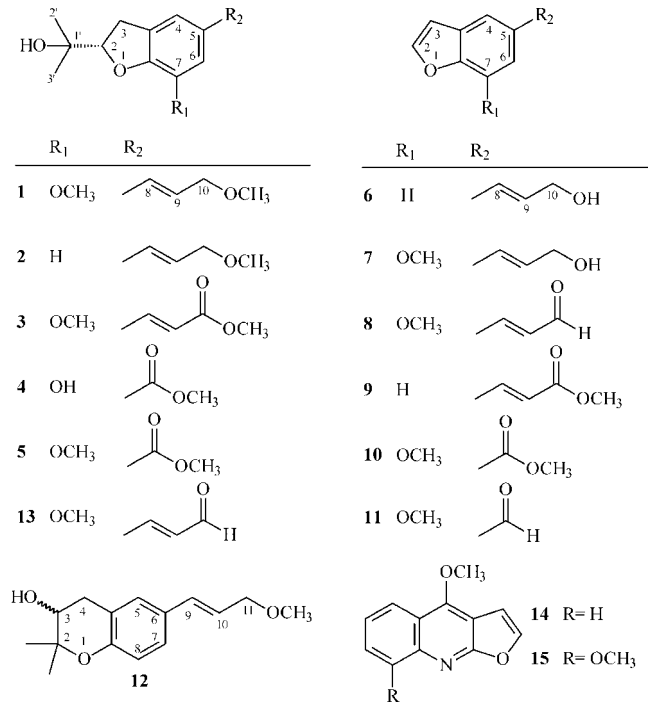
Bioassay-guided fractionation of the root wood of *Zanthoxylum wutaiense* led to the isolation of 11 new compounds, wutaiensol methyl ether (**1**), demethoxywutaiensol methyl ether (**2**), methyl wutaiensate (**3**), methyl 7-hydroxyanodendroate (**4**), methyl 7-methoxyanodendroate (**5**), wutaifuranol (**6**), 7-methoxywutaifuranol (**7**), 7-methoxywutaifuranal (**8**), methyl wutaifuranate (**9**), methyl 7-methoxybenzofuran-5-carboxylate (**10**), and wutaipyranol (**12**), together with another 37 known compounds, of which one, 7-methoxybenzofuran-5-carboxaldehyde (**11**), was not previously known as a plant constituent. The structures of these isolates were identified by means of spectroscopic analysis. Five of these isolates were found to be antitubercular constituents, namely, methyl 7-methoxyanodendroate (**5**), 7-methoxywutaifuranal (**8**), wutaiensal (**13**), dictamnine (**14**), and γ-fagarine (**15**), which exhibited antitubercular activity against *Mycobacterium tuberculosis* H37Rv, showing MIC values of 35, 35, 30, 30, and 30 μg/mL, respectively.

Zanthoxylum wutaiense Chen (Rutaceae) is an endemic evergreen shrub that grows in Pingtung County, Taiwan.[1] This plant was discovered and named by the corresponding author.[2] Four compounds, wutaiensol, wutaiensal, wutaialdehyde, and methyl demethoxywutaiensate, derived from the root wood of this species, have been reported previously.[3] In the past, the chemical constituents and antiplatelet aggregation activities of the Formosan *Zanthoxylum* plants, except for the species *Z. wutaiense*, have been studied extensively in our laboratory.[4] Recently, about 400 species of Formosan plants have been screened for antitubercular activity against *Mycobacterium tuberculosis* H37Rv, and *Z. wutaiense* was shown to be one of the active species. Among the different parts of this species, only the methanol extract of the root wood showed antitubercular activity against *Mycobacterium tuberculosis* H37Rv, with a MIC value of 21.2 μg/mL. Bioassay-guided investigation of the root wood of *Z. wutaiense* led to the isolation of 11 new compounds (**1**−**10**, **12**) and 37 known compounds. The isolation and structural elucidation of these compounds and an assessment of their in vitro antitubercular activities are described herein.

Chart 1



Results and Discussion

Compound **1** was isolated as a pale yellowish oil, and its molecular formula was established as $C_{16}H_{22}O_4$ by EIMS ([M]$^+$, *m*/*z* 278) and HREIMS. The UV absorption maxima at 232 and 268 nm suggested the presence of a benzenoid moiety, and the IR spectrum showed a hydroxyl group at 3428 cm^{-1}. The ^{1}H NMR spectrum of **1** was similar to that of wutaiensol,[3] isolated from this plant previously, except that a *trans*-3-hydroxy-1-propenyl group in wutaiensol was replaced by a *trans*-3-methoxy-1-propenyl group [δ 6.51 (1H, br d, J = 16.0 Hz, H-8), 6.11 (1H, dt, J = 16.0, 6.0 Hz, H-9), 3.36 (3H, s, OCH_3-10), 4.05 (2H, dd, J = 6.0, 1.4 Hz, H-10)] in **1**. Thus, compound **1** was proposed as a methyl ether of wutaiensol,[3] namely, wutaiensol methyl ether, which was confirmed by COSY, ^{13}C, DEPT, NOESY (Figure 1), and HMBC (Figure 1) NMR experiments. Compound **1** showed dextrorotatory optical activity with $[\alpha]^{24}_D$ +135.0 (*c* 0.04, $CHCl_3$). The specific rotation of 5-formyl-2-(2-hydroxyisopropyl)-2,3-dihydrobenzofuran with the *S* configuration at C-2 is also positive, suggesting that compound **1** also has an *S* configuration.[5]

Compound **2** was obtained as a yellow oil, and its molecular formula was established as $C_{15}H_{20}O_3$ by EIMS ([M]$^+$, *m*/*z* 248) and HREIMS analysis. The ^{1}H NMR spectrum of **2** was similar to that of **1**, except that one methoxyl group at C-7 [δ 3.87 (s)] in **1** was replaced by the H-7 proton signal at δ 6.71, resulting in an ABX system [δ 7.23 (1H, d, J = 1.2 Hz, H-4), 7.12 (1H, dd, J = 8.0, 1.2 Hz, H-6), 6.71 (1H, d, J = 8.0 Hz, H-7)] in the benzene ring of **2**. Thus, the structure of **2** was elucidated as demethoxywutaiensol methyl ether, which was confirmed by COSY, ^{13}C, HSQC, NOESY (Figure 1), and HMBC (Figure 1) NMR experiments. Compound **2** showed dextrorotatory optical activity, $[\alpha]^{24}_D$ +87.4 (*c* 0.03, $CHCl_3$), so the C-2 configuration of **2** may also be suggested to be in the *S* form.[5]

Compound **3** was obtained as colorless needles, and the molecular formula was determined as $C_{16}H_{20}O_5$ by EIMS ([M]$^+$, *m*/*z* 292)

* To whom correspondence should be addressed. Tel: (+886)-(0)7-312-1101, ext. 2191. Fax: (+886)-(0)7-321-0683. E-mail: m635013@kmu.edu.tw.
† College of Pharmacy, Kaohsiung Medical University.
‡ Chiba University.
§ College of Health Sciences, Kaohsiung Medical University.

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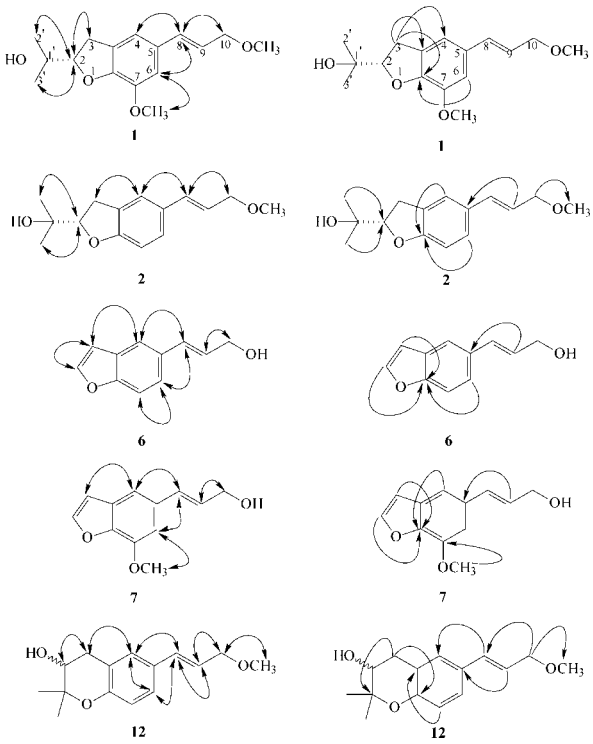

Figure 1. Key NOESY (↔) and HMBC connectivites (→) for compounds **1**, **2**, **6**, **7**, and **12**.

and HREIMS. A UV absorption maxima at 324 nm showed conjugation, and the IR spectrum revealed a hydroxyl group at 3499 cm^{-1} and an ester carbonyl group at 1713 cm^{-1}. The ^1H NMR spectrum was similar to that of methyl demethoxywutaiensate,[3] also isolated in this study, except that three aromatic proton signals [δ 6.71, 7.25 (each 1 H, d, $J = 8.5$ Hz, Ar H), 7.30 (1H, s, Ar H)] in methyl demethoxywutaiensate were replaced by a pair of *meta*-coupled protons [δ 7.04 (1H, br d, $J = 1.2$ Hz, H-4) and 6.94 (1H, br d, $J = 1.2$ Hz, H-6)] in **3**. Thus, **3** could be proposed as the methyl ester of the so far unreported wutaiensic acid and named methyl wutaiensate, which was further confirmed by 2D NMR spectroscopic experiments. Methyl wutaiensate showed a dextrorotarory optical activity as $[\alpha]^{24}_D$ +17.4 (*c* 0.02, $CHCl_3$), so the configuration of C-2 was proposed to be in the *S* form, the same as **1** and **2**.

Compound **4** was obtained as colorless needles, and its molecular formula was established as $C_{13}H_{16}O_5$ by EIMS ($[M]^+$, *m*/*z* 252) and HREIMS. The UV spectrum exhibited bands at 209, 228 (sh), 271, 304 (sh) nm, suggesting the presence of a conjugated dihydrobenzofuranoid moiety. Bands attributable to an ester carbonyl group (1694 cm^{-1}) and a hydroxyl group (3403 cm^{-1}) were observed in the IR spectrum. The ^1H and ^{13}C NMR spectra showed signals assignable to a pair of *meta*-coupled protons at δ 7.43 (1H, d, $J = 0.8$ Hz, H-4) and 7.45 (1H, d, $J = 0.8$ Hz, H-6), a phenolic hydroxyl group at δ 6.45 (1H, br s, OH-7, D_2O exchangeable), and a methyl carboxylate [δ 3.85 (3H, s, OCH_3-8), 167.0 (C-8)] in the benzene ring of the 2-(2-hydroxypropan-2-yl)dihydrobenzofuran unit. The HMBC spectrum showed correlations between H-4 (δ 7.43) and C-3 (δ 31.0) and C-8 (δ 167.0), suggesting the presence of a methyl carboxylate group at C-5 and a phenolic hydroxyl group at C-7. According to the above observations, the structure of compound **4** was elucidated as methyl 7-hydroxyanodendroate, which was confirmed by NOESY, HSQC, and HMBC NMR experiments.

The molecular formula of compound **5** was determined as $C_{14}H_{18}O_5$ by EIMS and HREIMS. The UV absorption maxima and IR for a hydroxyl group and an ester carbonyl group in **5** were similar to those in **4**, also suggesting the presence of a compound with an anodendroate skeleton. The ^1H NMR spectrum of **5** was similar to that of **4** except that a phenolic hydroxyl group at δ 6.45 (1H, s, OH-7) in **4** was observed instead of the methoxyl group at δ 3.90 (3H, s, OCH_3-7) in **5**. Thus, the structure of **5** was elucidated as methyl 7-methoxyanodendroate, which was supported by ^{13}C, HSQC, NOESY, and HMBC experiments. Compound **4** exhibited $[\alpha]^{24}_D$ +52.4 (*c* 0.07, $CHCl_3$), and compound **5**, $[\alpha]^{24}_D$ +31.7 (*c* 0.04, $CHCl_3$), respectively. Thus, the configurations of C-2 in compounds **4** and **5** could be suggested as *S* form, comparing with (−)-*R*-anodendroic acid, $[\alpha]^{15}_D$ −35.2 (*c* 0.082, EtOH).[6] In addition, methyl 7-methoxyanodendroate was obtained as a synthetic racemate, but no spectroscopic data have been reported.[3]

Compound **6** was obtained as a yellowish oil. Its molecular formula of $C_{11}H_{10}O_2$ was determined by EIMS ($[M]^+$, *m*/*z* 174) and HREIMS. The UV spectrum showed absorption maxima at 247, 262 (sh), 275 (sh), and 291 (sh) nm. The IR spectrum showed a hydroxyl group at 3417 cm^{-1}. The ^1H NMR spectrum showed two *ortho*-coupled protons characteristic of a furan ring, three protons of an ABX system at δ 7.60 (1H, br s, H-4), 7.38 (1H, br d, $J = 8.5$ Hz, H-6), and 7.45 (1H, d, $J = 8.5$ Hz, H-7), and a *trans*-3-hydroxy-1-propenyl group [δ 6.72 (1H, d, $J = 16.0$ Hz, H-8), 6.36 (1H, dt, $J = 16.0$, 5.5 Hz, H-9), 4.35 (2H, d, $J = 5.5$ Hz, H-10)]. The NOESY spectrum showed correlations between H-4 (δ 7.60) and H-3 (δ 6.76) and H-8 (δ 6.72) and was used to determine the location of the *trans*-3-hydroxy-1-propenyl group at C-5 in the benzofuran unit. On the basis of the above evidence, the structure of compound **6** was elucidated as wutaifuranol.

Compound **7** was also isolated as a yellowish oil, and a molecular formula of $C_{12}H_{12}O_3$ was established by EIMS and HREIMS. The UV and IR spectra of **7** were similar to those of **6**. The ^1H NMR spectrum of **7** was also similar to that of **6** except that two *meta*-coupled proton signals were observed at δ 7.21 (1H, br s, H-4) and 6.92 (1H, br s, H-6). In the HMBC spectrum, a methoxyl group signal at δ 4.05 (3H, s, OCH_3-7) in **7** replaced the three ABX-type protons in the benzene ring of the benzofuran unit in **6**. Thus, the structure of compound **7** was elucidated as 7-methoxywutaifuranol.

Compounds **8** and **9** have the same molecular formula of $C_{12}H_{10}O_3$ as deduced from their HREIMS and ^{13}C NMR data. The ^1H NMR data of **8** and **9** revealed the presence of a furan ring and one methoxyl group in common. From the HMBC spectrum, it was discerned that the methoxyl group (δ 4.06) of **8** is attached at C-7, and the methoxyl group (δ 3.82) of **9** was attached to the carbonyl group of C-10. Compound **8** showed one pair of *meta*-coupled protons at δ 7.43 (1H, d, $J = 1.2$ Hz, H-4) and 7.02 (1H, d, $J = 1.2$ Hz, H-6) and a *trans*-2-formylethenyl group [δ 7.56 (1H, d, $J = 16.0$ Hz, H-8), 6.71 (1H, dd, $J = 16.0$, 7.6 Hz, H-9), and 9.71 (1H, d, $J = 7.6$ Hz, CHO-9)], while **9** showed an ABX-type proton system at δ 7.74 (1H, d, $J = 1.0$ Hz, H-4), 7.50 (2H, br d, $J = 1.0$ Hz, H-6 and H-7) [7.69 (1H, dd, $J = 8.6$, 1.6 Hz, H-6 in acetone-d_6), and 7.59 (1H, d, $J = 8.6$ Hz, H-7 in acetone-d_6)], as well as a *trans*-methyl 1-propenoate group [δ 7.80 (1H, d, $J = 16.0$ Hz, H-8), 6.44 (1H, d, $J = 16.0$ Hz, H-9), 3.82 (3H, s, OCH_3-10)]. According to the data obtained, the structure of **8** was elucidated as 7-methoxywutaifuranal, while the structure of **9** was assigned as methyl wutaifuranate.

Compounds **10** and **11** were isolated as colorless needles, and their respective molecular formulas of $C_{11}H_{10}O_4$ and $C_{10}H_8O_3$ were established by EIMS and HREIMS. The ^1H NMR data (Table 3) of **10** and **11** revealed the presence of a furan ring, with one pair of aromatic *meta*-coupled protons in common. Moreover, compound **10** showed two methoxyls (δ 3.94, 4.06), while compound **11** exhibited one methoxyl (δ 4.07) and one aldehyde group (δ 10.0). From the HMBC NMR data, compounds **10** and **11** showed an attached methoxy group at C-7. Compound **10** gave evidence for a methyl carboxylate at δ 3.94 (3H, s, OCH_3-8), and compound **11** an aldehyde group at δ 10.0 (1H, s, C-5). On the basis of the data above, compound **10** was designated as methyl 7-methoxy-

benzofuran-5-carboxylate and **11** as 7-methoxybenzofuran-5-carboxaldehyde. This is the first time compound **11** has been isolated from a natural source, though it had been synthesized previously.[7]

Compound **12** was isolated as a colorless oil, and its molecular formula was established as $C_{15}H_{20}O_3$ by EIMS and HREIMS. The UV absorption maxima at 208 and 266 nm indicated the presence of a benzenoid moiety, and the IR spectrum showed a hydroxyl group at 3445 cm^{-1}. The ^1H NMR data of **12** were similar to those of phebarudol,[10] also isolated in this study, except for the presence of a *trans*-3-methoxy-1-propenyl group [δ 6.51 (1H, br d, $J = 16.0$ Hz, H-9), 6.12 (1H, dt, $J = 16.0$, 6.1 Hz, H-10), and 4.06 (2H, dd, $J = 6.1$, 1.4 Hz, H-11)] in **12** in place of the *trans*-3-methoxy-2-oxy-1-propenyl group [δ 7.61 (1H, d, $J = 16.0$ Hz, H-9), 6.28 (1H, d, $J = 16.0$ Hz, H-10), and 3.78 (3H, s, OCH_3-11) in phebarudol. Three aromatic protons in an ABX system [δ 6.78 (1H, d, $J = 8.4$ Hz), 7.09 (1H, d, $J = 2.0$ Hz), 7.18 (1H, dd, $J = 8.4$, 2.0 Hz)], the presence of a 2,2-dimethyl-3-hydroxydihydropyran moiety [δ 2.77 (1H, dd, $J = 16.8$, 5.6 Hz, H-4α), 3.06 (1H, dd, $J = 16.8$, 4.8 Hz, H-4β), 3.78 (1H, dd, $J = 5.6$, 4.8 Hz, H-3), 1.31, 1.36 (each 3H, s, CH_3-2)], and NOESY correlations between H-5 (δ 7.09) and H-4 (δ 2.77, 3.06), H-9 (δ 6.51) and betweeen H-9 (δ 6.51) and H-5 (δ 6.51), H-7 (δ 7.18) permitted structure **12** to be elucidated as wutaipyranol, which was supported by ^{13}C NMR, NOESY (Figure 1), and HMBC (Figure 1) experiments. The absolute configuration of C-3′ remains uncertain, due to the small amount of **12** isolated.

The known compounds, methyl demethoxywutaiensate,[3] wutaiensal (**13**),[3] 5-formyl-2-(2-hydroxyisopropyl)-2,3-dihydrobenzofuran,[5] methyl anodendroate,[8] 5-(3-hydroxypropyl)-7-methoxybenzofuran,[9] phebarudol,[10] dictamnine (**14**),[11] robustine,[12] γ-fagarine (**15**),[11] skimmianine,[11] *N*-methylflindersine,[13] atanine,[14] rutaecarpine,[15] 1-hydroxylrutaecarpine,[16] umbelliferone,[17] 3-[4-(3-methyl-2-butenyloxy)phenyl]-1-propanol,[18] 3-[4-(3-methylbut-2-enyloxy)phenyl]-acrylic acid methyl ester,[19] methyl ferulate,[20] valencic acid,[21] 4-hydroxy-3-prenylbenzoic acid,[22] 4-hydroxybenzaldehyde,[23] vanillin,[24] syringaldehyde,[23] ligballinol,[25] (−)-xanthoxylol,[26] (−)-xanthoxylol-3,3-dimethylallyl ether,[27] a mixture of campesterol and β-sitosterol,[11,28,29] a mixture of campest-4-en-3-one and β-sitostenone,[30] a mixture of 3β-hydroxycampest-5-en-7-one and 3β-hydroxystigmast-5-en-7-one,[31] a mixture of 6α-hydroxycampest-4-en-3-one and 6α-hydroxystigmast-4-en-3-one,[30] and a mixture of 6β-hydroxylcampest-4-en-3-one and 6β-hydroxystigmast-4-en-3-one,[30] were identified in each case by comparison of their physical and spectroscopic data ($[\alpha]_D$, UV, IR, ^1H NMR, and MS) with values found in the literature.

Zanthoxylum species are well known for the benzo[*c*]phenanthridine alkaloid constituents in the bark or root bark. Benzo[*c*]phenanthridine alkaloids have also been detected in small amounts in the root woods of some Formosan *Zanthoxylum* species, such as *Z. integrifoliolum*[32] and *Z. simulans*.[33] It is interesting that metabolites of the benzofuran and the dihydrobenzofuran types were found in the root wood of *Z. wutaiense*. Only four beznzofurans, 6-methoxybenzofuran-5-propionyl methyl ester,[34] 6,7-dimethoxybenzofuran-5-propionyl methyl ester,[34] 5-(3-hydroxypropyl)-7-methoxybenzofuran,[9] and wutaiensal (**13**),[35] have ever been isolated from other *Zanthoxylum* species.

Bioactivity-guided fractionation of the active $CHCl_3$ fraction of *Z. wutaiense* led to the isolation of **5**, **8**, **13**, **14**, and **15** as active constituents with antitubercular activities against *M. tuberculosis* H37Rv, showing MIC values of 35, 35, 30, 30, and 30 μg/mL, respectively (Table 1). Ethambutol (MIC 6.25 μg/mL) was used as the positive control. From the results of our antitubercular tests, this revealed that the carbonyl group of benzofurans and dihydrobenzofurans probably plays an important role in mediating the antitubercular activity.

Table 1. Antitubercular Activities of Isolates from the Root Wood of *Zanthoxylum wutaiense* on *M. tuberculosis* H37Rv

compound	MIC (μg/mL)
methyl 7-methoxyanodendroate (**5**)	35
7-methoxywutaifuranal (**8**)	35
methyl demethoxywutaiensate	50
wutaiensal (**13**)	30
5-(3-hydroxypropyl)-7-methoxybenzofuran	50
dictamnine (**14**)	30
γ-fagarine (**15**)	30
valencic acid	45
ethambutol[a]	6.25

[a] Positive control.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanaco micromelting point apparatus and are uncorrected. Optical rotations were measured using a JASCO P-1020 polarimeter. UV spectra were measured in MeOH on a JASCO UV-240 spectrophotometer. IR spectra (KBr disc or neat) were taken on a Perkin-Elmer System 2000 FT-IR spectrometer. The NMR spectra were recorded on a Varian Unity-plus 400 or a Varian Unity Inova-500 MHz NMR spectrometer. Chemical shifts are given in ppm (δ) with TMS as an internal standard. EIMS were recorded on a Micromass TRIO-2000 GC/MS spectrometer, and HREIMS were recorded on a Finnigan/Thermo Quest MAT mass spectrometer. Silica gel (70−230 or 230−400 mesh) (Merck) was used for column chromatography, and silica gel 60 F-254 (Merck) was used for TLC and preparative TLC.

Plant Material. The root wood of *Z. wutaiense* was collected from Wutai, Pingtung County, Taiwan, in August 2004 and identified by one of us (I.-S.C.). A voucher specimen (Chen 2512) has been deposited in the herbarium of the School of Pharmacy, Kaohsiung, Taiwan, Republic of China.

Extraction and Isolation. The dried root wood (10.4 kg) of *Z. wutaiense* was extracted with cold MeOH (65 L) three times, and the extract concentrated under reduced pressure. The methanolic extract (394 g) was partitioned into $CHCl_3$- (fraction A, 80 g), EtOAc- (fraction B, 19 g), *n*-BuOH- (fraction C, 71 g), and water-soluble (fraction D, 197 g) fractions. Fraction A (79 g) was subjected to silica gel column chromatography (2.5 kg), eluting with *n*-hexane and gradually enriching with EtOAc, acetone, and MeOH to afford 13 fractions. There were fractions A1 (10 L, *n*-hexane), A2 (7.5 L, *n*-hexane−EtOAc, 20:1), A3 (11 L, *n*-hexane−EtOAc, 10:1), A4 (15 L, *n*-hexane−EtOAc, 5:1), A5 (5 L, *n*-hexane−EtOAc, 2:1), A6 (12 L, *n*-hexane−EtOAc, 2:1), A7 (15 L, *n*-hexane−EtOAc, 1:1), A8 (4 L, EtOAc), A9 (9 L, EtOAc), A10 (6 L, EtOAc), A11 (2 L, acetone), A12 (10 L, acetone), and A13 (3 L, MeOH). Fraction A3 (4.8 g) was chromatographed on silica gel (145 g), eluting with *n*-hexane−EtOAc, to furnish five fractions. Fraction A-3-2 (92 mg) was purified by preparative TLC (*n*-hexane−acetone, 10:1) to yield **3** (3.5 mg, R_f 0.46) and **10** (1.9 mg, R_f 0.41). Fraction A-3-4 (116 mg) was purified by preparative TLC (*n*-hexane−acetone, 8:1) to yield **9** (6.1 mg, R_f 0.64). Fraction A4 (7.5 g) was triturated with MeOH, and the soluble material (5.9 g) was subjected to silica gel column chromatography (176 g), with the purification accomplished by preparative TLC (*n*-hexane−EtOAc, 10:1), to furnish methyl ferulate (1.1 mg, R_f 0.53). Fraction A5 (6.3 g, *n*-hexane−EtOAc, 2:1) was triturated with MeOH to obtain an insoluble mixture (2.8 g, R_f 0.83) of campesterol and β-sitosterol. Fraction A6 (0.4 g) was applied to a silica gel column (12 g), eluting with *n*-hexane−acetone (5:1), to give seven fractions. Each subfraction was purified by preparative TLC (*n*-hexane−EtOAc, 10:1) to yield (−)-xanthoxylol-3,3-dimethylallyl ether (7.8 mg, R_f 0.54), 3-[4-(3-methyl-2-butenyloxy)phenyl]-1-propanol (5.2 mg, R_f 0.48), 5-(3-hydroxypropyl)-7-methoxybenzofuran (15.3 mg, R_f 0.21), 5-formyl-2-(2-hydroxyisopropyl)-2,3-dihydrobenzofuran (9.3 mg, R_f 0.26), phebarudol (3.4 mg, R_f 0.24), 4-hydroxybenzaldehyde (3.4 mg, R_f 0.33), and (−)-xanthoxylol (1.9 mg, R_f 0.27). Fraction A7 (6.8 g) was subjected to passage over silica gel (230−400 mesh, 204 g), eluting with *n*-hexane−acetone (5:1), to yield a further nine fractions. Fraction A-7-2 (0.8 g, *n*-hexane−acetone, 10:1) was purified by preparative TLC (*n*-hexane−acetone, 10:1) to obtain a mixture (217 mg, R_f 0.53) of campest-4-en-3-one and β-sitostenone. Fraction A-7-3 (0.7 g, *n*-hexane−acetone, 8:1) was also further purified by preparative TLC ($CHCl_3$−acetone, 30:1) to furnish robustine (6.7 mg, R_f 0.76) and syringaldehyde (7.1 mg, R_f 0.63). Fraction A-7-4 (1.1 g, *n*-hexane−acetone, 5:1) was applied to a silica gel column (33 g), eluting with *n*-hexane−acetone (5:1), to obtain eight fractions. Each subfraction was further purified by preparative TLC

Table 2. ^{1}H NMR Data of Compounds **1**−**5** (δ in ppm, J in Hz)

position	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]
2	4.66 (t, 9.2)	4.62 (t, 9.2)	4.76 (t, 8.8)	4.75 (t, 9.0)	4.74 (t, 9.2)
3α	3.11 (dd, 15.6, 9.2)	3.11 (dd, 15.8, 9.2)	3.17 (dd, 15.6, 8.8)	3.14 (dd, 15.4, 9.0)	3.16 (dd, 16.0, 9.2)
3β	3.16 (dd, 15.6, 9.2)	3.17 (dd, 15.8, 9.2)	3.23 (dd, 15.6, 8.8)	3.23 (dd, 15.4, 9.0)	3.21 (dd, 16.0, 9.2)
4	6.84 (br s)	7.23 (d, 1.2)	7.04 (br d, 1.2)	7.43 (d, 0.8)	7.52 (d, 1.0)
6	6.78 (br s)	7.12 (dd, 8.0, 1.2)	6.94 (br d, 1.2)	7.45 (d, 0.8)	7.45 (d, 1.0)
7		6.71 (d, 8.0)			
8	6.51 (br d, 16.0)	6.51 (br d, 16.0)	7.64 (d, 16.0)		
9	6.11 (dt, 16.0, 6.0)	6.11 (dt, 16.0, 6.2)	6.31 (d, 16.0)		
10	4.05 (dd, 6.0, 1.4)	4.05 (dd, 6.2, 0.6)			
2′	1.20 (s)	1.21 (s)	1.26 (s)	1.20 (s)	1.22 (s)
3′	1.35 (s)	1.34 (s)	1.42 (s)	1.35 (s)	1.37 (s)
OH-2′	2.15 (br s)	2.15 (br s)	1.62 (s)	2.01 (br s)	1.93 (br s)
OH-7				6.45 (br s)	
OCH_3-7	3.87 (s)		3.93 (s)		3.90 (s)
OCH_3-8				3.85 (s)	3.87 (s)
OCH_3-10	3.36 (s)	3.37 (s)	3.83 (s)		

[a] Recorded in $CDCl_3$ at 400 MHz.

Table 3. ^{1}H NMR Data of Compounds **6**−**11** (δ in ppm, J in Hz)

position	**6**[a]	**7**[b]	**8**[c]	**9**[b]	**9**[d]	**10**[c]	**11**[c]
2	7.61 (d, 2.0)	7.63 (d, 2.2)	7.68 (d, 2.4)	7.65 (d, 2.2)	7.91 (d, 2.2)	7.68 (d, 2.4)	7.73 (d, 2.4)
3	6.76 (d, 2.0)	6.76 (d, 2.2)	6.81 (d, 2.4)	6.79 (d, 2.2)	6.96 (d, 2.2)	6.83 (d, 2.4)	6.90 (d, 2.4)
4	7.60 (br s)	7.21 (br s)	7.43 (d, 1.2)	7.74 (d, 1.0)	7.99 (d, 1.6)	7.98 (d, 1.2)	7.75 (d, 1.2)
6	7.38 (br d, 8.5)	6.92 (br s)	7.02 (d, 1.2)	7.50 (br d, 1.0)	7.69 (dd, 8.6, 1.6)	7.52 (d, 1.2)	7.38 (d, 1.2)
7	7.45 (d, 8.5)			7.50 (br d, 1.0)	7.59 (d, 8.6)		
8	6.72 (d, 16.0)	6.72 (br d, 16.0)	7.56 (d, 16.0)	7.80 (d, 16.0)	7.79 (d, 16.0)		
9	6.36 (dt, 16.0, 5.5)	6.36 (dt, 16.0, 6.0)	6.71 (dd, 16.0, 7.6)	6.44 (d, 16.0)	6.55 (d, 16.0)		
10	4.35 (d, 5.5)	4.27 (d, 6.0)					
OCH_3-7		4.05 (s)	4.06 (s)			4.06 (s)	4.07 (s)
OCH_3-8						3.94 (s)	
OCH_3-10				3.82 (s)	3.76 (s)		10.0 (s)
CHO			9.71 (d, 7.6)				

[a] Recorded in $CDCl_3$ at 500 MHz. [b] Recorded in $CDCl_3$ at 200 MHz. [c] Recorded in $CDCl_3$ at 400 MHz. [d] Recorded in acetone-d_6 at 200 MHz.

(*n*-hexane−EtOAc, 10:1) to afford vanillin (5.1 mg, R_f 0.24), **6** (1.59 mg, R_f 0.25), **14** (8.9 mg, R_f 0.21), rutaecarpine (1.5 mg, R_f 0.23), 1-hydroxylrutaecarpine (0.9 mg, R_f 0.21), *N*-methylflindersine (6.3 mg, R_f 0.51), **2** (3.5 mg, R_f 0.46), methyl demethoxywutaiensate (15.8 mg, R_f 0.44), methyl anodendroate (4.4 mg, R_f 0.42), **12** (1.8 mg, R_f 0.39), and a mixture (15.4 mg, R_f 0.43) of 6β-hydroxylcampest-4-en-3-one and 6β-hydroxystigmast-4-en-3-one. Fraction A-7-5 (2.1 g, *n*-hexane−acetone, 5:1) was chromatographed on silica gel (63 g), eluting with *n*-hexane−acetone (5:1), to give nine fractions. Each subfraction was purified by preparative TLC (*n*-hexane−EtOAc, 10:1) to produce **8** (6.5 mg, R_f 0.35), atanine (2.6 mg, R_f 0.32), **1** (9.1 mg, R_f 0.47), a mixture (10.4 mg, R_f 0.56) of 3β-hydroxycampest-5-en-7-one and 3β-hydroxystigmast-5-en-7-one, a mixture (7.3 mg, R_f 0.52) of 6α-hydroxycampest-4-en-3-one and 6α-hydroxystigmast-4-en-3-one, **5** (12.0 mg, R_f 0.26), 3-[4-(3-methylbut-2-enyloxy)phenyl]acrylic acid methyl ester (2.4 mg, R_f 0.34), umbelliferone (6.5 mg, R_f 0.32), **11** (1.1 mg, R_f 0.29), **7** (7.9 mg, R_f 0.34), and valencic acid (7.5 mg, R_f 0.21). Fraction A-7-6 (1.1 g, *n*-hexane−acetone, 3:1) was subjected to silica gel chromatography (30 g) and purified by preparative TLC (*n*-hexane−EtOAc, 10:1) to obtain 4-hydroxy-3-prenylbenzoic acid (5.6 mg, R_f 0.33). Finally, fraction A-7-7 (0.7 g, *n*-hexane−acetone, 3:1) was subjected to silica gel chromatography (19 g), eluting with *n*-hexane−acetone (5:1), to give six fractions. Each subfraction was purified by preparative TLC (*n*-hexane−EtOAc, 10:1) to give **15** (5.9 mg, R_f 0.27), skimmianine (3.1 mg, R_f 0.24), **13** (9.3 mg, R_f 0.22), **4** (6.1 mg, R_f 0.23), and ligballinol (2.1 mg, R_f 0.31).

Wutaiensol methyl ether (1): pale yellow oil; $[\alpha]^{24}_D$ +135 (*c* 0.04, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 232 (4.43), 268 (4.15) nm; IR (neat) ν_{max} 3428 (OH) cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 2 and 4; EIMS *m*/*z* 278 $[M]^+$ (100), 189 (18), 157 (12), 84 (34); HREIMS *m*/*z* 278.1516 (calcd for $C_{16}H_{22}O_4$, 278.1514).

Demethoxywutaiensol methyl ether (2): yellow oil; $[\alpha]^{24}_D$ +87.4 (*c* 0.03, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 206 (4.37), 237 (sh) (3.86), 272 (3.72) nm; IR (neat) ν_{max} 3417 (OH) cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 2 and 4; EIMS *m*/*z* 248 $[M]^+$ (100), 206 (17), 178 (40), 147 (46), 119 (34); HREIMS *m*/*z* 248.1413 (calcd for $C_{15}H_{20}O_3$, 248.1414).

Methyl wutaiensate (3): colorless needles (CH_2Cl_2−MeOH); mp 87−91 °C; $[\alpha]^{24}_D$ +17.4 (*c* 0.02, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 206 (3.55), 222 (3.97), 244 (sh) (3.76), 324 (4.03) nm; IR (KBr) ν_{max} 3499 (OH), 1713 (C=O) cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 2 and 4; EIMS *m*/*z* 292 $[M]^+$ (100), 259 (14), 234 (62), 221 (23), 202 (42), 190 (14), 178 (27), 174 (16), 147 (32), 131 (14); HREIMS *m*/*z* 292.1312 (calcd for $C_{16}H_{20}O_5$, 292.1313).

Methyl 7-hydroxyanodendroate (4): colorless needles ($CHCl_3$−MeOH); mp 89−92 °C; $[\alpha]^{22}_D$ +52.4 (*c* 0.07, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 209 (4.07), 228 (sh) (4.17), 271 (4.13), 304 (sh) (3.58) nm; IR (KBr) ν_{max} 1694 (C=O), 3403 (OH) cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 2 and 4; EIMS *m*/*z* 252 $[M]^+$ (81), 221 (29), 194 (100), 181 (32), 163 (34), 161 (30), 149 (25),134 (40), 107 (22), 59 (54); HREIMS *m*/*z* 252.1006 (calcd for $C_{13}H_{16}O_5$, 252.1014).

Methyl 7-methoxyanodendroate (5): colorless prisms ($CHCl_3$−MeOH); mp 84−87 °C; $[\alpha]^{24}_D$ +31.7 (*c* 0.04, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 209 (3.96), 226 (sh) (4.21), 272 (4.17), 300 (sh) (3.63) nm; IR (KBr) ν_{max} 1712 (C=O), 3481 (OH) cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 2 and 4; EIMS *m*/*z* 266 $[M]^+$ (57), 237 (36), 208 (100), 177 (39), 149 (50); HREIMS *m*/*z* 266.1156 (calcd for $C_{14}H_{18}O_5$, 266.1157).

Wutaifuranol (6): yellowish oil; UV (MeOH) λ_{max} (log ϵ) 247 (4.17), 262 (sh) (3.83), 275 (sh) (3.72), 291 (sh) (3.55) nm; IR (neat) ν_{max} 3417 (OH) cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 3 and 4; EIMS *m*/*z* 174 $[M]^+$ (100), 145 (23), 131 (90), 118 (64); HREIMS *m*/*z* 174.0681 (calcd for $C_{11}H_{10}O_2$, 174.0681).

7-Methoxywutaifuranol (7): yellowish oil; UV (MeOH) λ_{max} (log ϵ) 245 (4.42), 274 (sh) (3.79), 294 (sh) (3.63) nm; IR (neat) ν_{max} 3404 (OH) cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 3 and 4; EIMS *m*/*z* 204 $[M]^+$ (100), 175 (43), 161 (49), 148 (22); HREIMS *m*/*z* 204.0785 (calcd for $C_{12}H_{12}O_3$, 204.0784).

7-Methoxywutaifuranal (8): colorless prisms (CH_2Cl_2−MeOH); mp 93−95 °C; UV (MeOH) λ_{max} (log ϵ) 251 (3.87), 263 (sh) (3.51), 309 (3.79) nm; IR (KBr) ν_{max} 1670 (C=O) cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Tables 3 and 4; EIMS *m*/*z* 202 $[M]^+$ (100), 174 (61), 159(19), 131 (34); HREIMS *m*/*z* 202.0269 (calcd for $C_{12}H_{10}O_3$, 202.0268).

Table 4. ^{13}C NMR Data of Compounds **1**−**11** (chemical shifts are in ppm)

position	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]	**6**[b]	**7**[c]	**8**[a]	**9**[c]	**10**[a]	**11**[a]
2	90.6	89.6	90.9	91.0	91.1	145.5		146.2	146.0	146.1	146.5
3	31.0	30.6	30.7	31.0	30.6	106.6	107.0	107.2	106.8	107.5	107.6
3a	128.5	127.6	128.2	128.0	128.3	127.8	129.3	129.4	128.0	128.7	129.0
4	115.6	122.7	117.9	118.8	119.4	119.2	112.2	115.9	121.6	116.4	119.9
5	130.5	129.7	129.9	123.6	123.3	131.7	132.9	130.2	129.5	126.0	133.5
6	109.4	129.7	111.0	117.4	112.7	122.9	104.6	105.3	124.1	107.2	104.2
7	143.9	109.0	144.3	139.7	143.7	111.4	145.4	145.9	111.9	145.1	146.3
7a	147.7	159.4	150.2	150.8	152.0	154.7		146.2	156.0	147.0	148.1
8	132.7	132.0	145.0	167.0 (C═O)	166.9 (C═O)	131.6	131.8	153.7	145.3	167.3 (C═O)	191.8 (CHO)
9	123.5	123.1	115.0			127.3	127.5	127.7	116.6		
10	73.2	73.3	167.0 (C═O)			63.9	63.8	193.6 (CHO)	167.6 (C═O)		
1′	71.6	71.8	71.7	72.2	71.6						
2′	24.0	23.9	24.1	23.4	24.0						
3′	26.1	26.1	26.2	25.9	25.9						
OCH_3-7	55.8		55.9				56.1	56.2		56.2	56.2
OCH_3-8				52.0	51.9					52.2	
OCH_3-10	57.8	57.8	51.6						51.6		

[a] Recorded in $CDCl_3$ at 100 MHz. [b] Recorded in $CDCl_3$ at 125 MHz. [c] Recorded in $CDCl_3$ at 50 MHz.

Methyl wutaifuranate (9): colorless needles (CH_2Cl_2−MeOH); mp 91−94 °C; UV (MeOH) λ_{max} (log ϵ) 250 (3.78), 288 (3.62), 313 (sh) (3.15) nm; IR (KBr) ν_{max} 1717 (C═O) cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 3 and 4; EIMS *m/z* 202 $[M]^+$ (92), 171 (100), 143 (28), 115 (37); HREIMS *m/z* 202.0631 (calcd for $C_{12}H_{10}O_3$, 202.0630).

Methyl 7-methoxybenzofuran-5-carboxylate (10): colorless needles ($CHCl_3$−MeOH); mp 78−81 °C; UV (MeOH) λ_{max} (log ϵ) 224 (4.01), 254 (sh) (3.53), 304 (3.22) nm; IR (KBr) ν_{max} 1714 (C═O) cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 3 and 4; EIMS *m/z* 206 $[M]^+$ (98), 175 (100), 147 (47); HREIMS *m/z* 206.0580 (calcd for $C_{11}H_{10}O_4$, 206.0581).

Methoxybenzofuran-5-carboxaldehyde (11): colorless needles ($CHCl_3$−MeOH); mp 79−83 °C; UV (MeOH) λ_{max} (log ϵ) 232 (4.01), 272 (3.32), 317 (sh) (3.17) nm; IR (KBr) ν_{max} 1693 (C═O) cm^{-1}; ^1H NMR and ^{13}C NMR, see Tables 3 and 4; EIMS *m/z* 176 $[M]^+$ (100), 147 (37), 105 (10), 77 (10); HREIMS *m/z* 176.0473 (calcd for $C_{10}H_8O_3$, 176.0473).

Wutaipyranol (12): colorless oil; $[\alpha]^{24}_D$ +34.2 (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 208 (4.37), 266 (4.01) nm; IR (neat) ν_{max} 3445 (OH) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.31 (3H, s, CH_3-2), 1.36 (3H, s, CH_3-2), 2.77 (1H, dd, J = 16.8, 5.6 Hz, H-4α), 3.06 (1H, dd, J = 16.8, 4.8 Hz, H-4β), 3.37 (3H, s, OCH_3-11), 3.78 (1H, dd, J = 5.6, 4.8 Hz, H-3), 4.06 (2H, dd, J = 6.1, 1.4 Hz, H-11), 6.12 (1H, dt, J = 16.0, 6.1 Hz, H-10), 6.51 (1H, br d, J = 16.0 Hz, H-9), 6.78 (1H, d, J = 8.4 Hz, H-8), 7.09 (1H, br d, J = 2.0 Hz, H-5), 7.18 (1H, dd, J = 8.4, 2.0 Hz, H-7); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.3 (CH_3-2), 24.7 (CH_3-2), 31.3 (C-4), 57.8 (OCH_3-11), 69.6 (C-3), 73.2 (C-11), 77.3 (C-2), 117.4 (C-8), 118.7 (C-4a), 123.6 (C-10), 125.9 (C-7), 128.3 (C-5), 129.5 (C-6), 132.2 (C-9), 152.6 (C-8a); EIMS *m/z* 249 $[M + 1]^+$ (17), 248 $[M]^+$ (100), 230 (11), 216 (14), 178 (16), 177 (24), 147 (22), 146 (56), 145 (68), 131 (21), 117 (32), 115 (26); HREIMS *m/z* 174.0681 (calcd for $C_{15}H_{20}O_3$, 174.0681).

Antitubercular Activity Assay. The in vitro antitubercular activity of each tested compound was evaluated using *Mycobacterium tuberculosis* H37Rv. Middlebrook 7H10 agar was used to determine MIC values, as recommended by the proportion method.[36] Briefly, each test compound was added to Middlebrook 7H10 agar supplemented with OADC (oleic acid−albumin−dextrose−catalase) at 50−56 °C by 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 a 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 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 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 inhibited the growth of the test isolate of *M. tuberculosis*, as detected by the unaided eye.

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