# Cytotoxic and Antibacterial Sesquiterpenes from *Thespesia populnea*

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Eight new sesquiterpenoids, named populene A−H (**1**−**8**), were isolated from dichloromethane extracts of the wood and dark heartwood of *Thespesia populnea*, together with 11 known compounds (**9**−**19**). Their structures were determined on the basis of spectroscopic analyses. The cytotoxic activity of isolated compounds was evaluated against four cancer cell lines: MCF-7, HeLa, HT-29, and KB. Mansonone E (**11**) and (+)-gossypol (**18**) showed significant activities. Their antibacterial properties against *Bacillus subtilis*, *Staphylococcus aureus*, and *Enterococcus faecalis* are also presented.

*Thespesia populnea* (L.) Soland. Ex Coor (Malvaceae) is widely distributed in Hawaii, California, Florida, Africa, the Caribbean islands, and Asia.[1] Various parts of this plant are found to possess useful medicinal properties, such as antifertility, antibacterial, anti-inflammatory, antioxidant, purgative, and hepatoprotective activities.[2] Previous chemical investigations of this species have yielded highly oxidized sesquiterpenes containing a cadinane skeleton.[1,3,4] Some of these compounds possess significant cytotoxic,[5−7] antifungal,[8] or antioxidative activities.[9]As part of our search for bioactive natural products from mangroves and tropical plants,[10−12] we now describe the isolation and structure elucidation of compounds **1**−**19** from the dark heartwood and wood of *T. populnea*. Two new compounds, populenes A and B (**1** and **2**), along with mansonone E (**11**),[5] (+)-gossypol (**18**),[13] and (+)-6,6′-methoxygossypol (**19**)[14] were purified from the wood. Six new compounds, populene C−H (**3**−**8**), were obtained from the dark heartwood, together with mansonones C,[5] D,[3] E,[5,9] G,[3] H,[9] and S[15] (**9**−**14**), 7-hydroxycadalene (**15**),[3] 7-hydroxy-2,3,5,6-tetrahydro-3,6,9-trimethylnaphtho[1,8-*b*,*c*]pyran-4,8-dione (**16**),[1,16] and thespesone (**17**).[1,3] Antibacterial and cytotoxic activities of **1**−**19** were also evaluated.

## Results and Discussion

The heartwood and wood of *T. populnea* were separately extracted with dichloromethane. These extracts showed significant cytotoxicity against MCF-7, HeLa, HT-29, and KB cancer cell lines and weak antibacterial activity against *B. subtilis*. Therefore, each extract was subjected to chromatography on silica gel to give compounds **1**−**19**.

Compound **1** was obtained as a yellow gum with the molecular formula $C_{15}H_{18}O_3$ on the basis of a molecular ion at *m*/*z* 246.1262 in the HREIMS. The IR spectrum of **1** showed a OH absorption (3365 $cm^{-1}$), and the UV spectrum showed absorption maxima at 216, 251, 259 (sh), 279, and 289, suggesting a benzofuran chromophore.[17] The $^1H$ NMR data of **1** (Table 1) were characteristic of a cadinane skeleton[1,3,8] with a benzofuran moiety. Aromatic protons resonating at $\delta$ 7.02 (1H, br s) and 7.10 (1H, br s) were assigned to H-4 and H-2, respectively, and a furan proton signal at $\delta$ 7.50 (d, $J$ = 0.9 Hz) was assigned to H-9. Signals assigned to a methine proton [$\delta$ 3.02 (dd, $J$ = 7.8, 3.9 Hz)], two oxymethines [$\delta$ 4.01 (dd, $J$ = 7.8, 7.8 Hz) and 4.90 (dd, $J$ = 7.8, 0.9 Hz)], a methyl group ($\delta$ 2.48, s) and one isopropyl moiety [$\delta$ 1.16 (d, $J$ = 7.2 Hz); 1.18 (d, $J$ = 7.2 Hz) and 2.58 (m)] were also observed. The methyl signal at $\delta$ 2.48 was placed at C-3 because of HMBC correlations to C-2 ($\delta$ 109.3) and C-4 ($\delta$ 121.6), and the isopropyl group was placed at C-5 due to HMBC correlations of the methine H-11 at $\delta$ 2.58 with C-4a ($\delta$ 131.4), C-5 ($\delta$ 49.8), and C-6 ($\delta$ 75.7). The two oxymethine protons at $\delta$ 4.01 and 4.90 were assigned to H-6 and H-7, respectively, judging from the allylic coupling (0.9 Hz) of H-9 with H-7, which was in turn coupled to H-6 in the COSY experiment. The relative configuration at C-5, C-6, and C-7 was assigned by NOESY experiment, in which only methyl protons of the isopropyl group showed a cross-peak with H-6, indicating that H-6 was on the same side of the ring as the isopropyl group but opposite that of H-5 and H-7. The pseudo-*trans*-diaxial coupling (7.8 Hz) of H-6 with H-5 and H-7 also supported the NOESY data. Therefore, the relative configuration at H-5, H-6, and H-7 should be *trans*−*trans*. Thus, **1** was named populene A.

Compound **2** possessed the same formula as **1** by HREIMS. The similarity of the mass, IR, UV, and $^1H$ and $^{13}C$ NMR spectra (Table 1) of **1** and **2** indicated that **2** was a diastereomer of **1**. The main difference was found in the small coupling constant of H-6 ($\delta$ 4.38, dd, $J$ = 3.3, 3.3 Hz) in **2** as compared to that in **1** ($\delta$ 4.01, t, $J$ = 7.8 Hz). The NOESY experiment exhibited cross-peaks of H-5 and H-6 and between H-6 and H-7, suggesting their *cis* orientation. Accordingly, the structure of **2** was as indicated, and it was named populene B.

Compound **3** had the molecular formula $C_{18}H_{20}O_3$ as determined by HREIMS. The EI mass spectrum was diagnostic, showing a relatively intense $[M + 2]^+$ ion peak characteristic of *ortho*-naphthoquinones, which was not displayed by *para*- naphthoquinones.[18] The IR spectrum exhibited carbonyl absorptions at 1757 and 1698 $cm^{-1}$. The UV spectrum showed absorption maxima at 213, 242, 259, and 380 nm. The $^1H$ and $^{13}C$ NMR data (Table 1) of **3** were comparable to those of mansonone D[3] (**10**), which was isolated from the dark heartwood of this plant. The difference between these two compounds was that **3** contained an additional isopropyl group, which appeared as two methyl singlet signals at $\delta$ 1.57 and 1.53 in the $^1H$ NMR spectrum. HMBC correlations to the oxygenated quaternary carbon at $\delta$ 74.9 (C-14) supported the connection of this group to oxygen. In addition, the correlation of oxymethylene protons at $\delta$ 3.97 and 3.79 ($H_2$-13) with C-5 ($\delta$ 135.8) and C-14 ($\delta$ 74.9), of a *gem*-dimethyl with C-6 ($\delta$ 150.1), and of aromatic proton H-7 ($\delta$ 6.95) with C-14 ($\delta$ 74.9) indicated that a pyran moiety was connected to an aromatic ring at C-5 and C-6. The methine proton on C-11 was deduced to be equatorially oriented from the two small vicinal coupling constants ($J_{11,13\beta}$ = 1.2 Hz and $J_{11,13\alpha}$ = 2.4 Hz). Therefore, compound **3** was named populene C.

Compound **4** was a brown gum having the molecular formula $C_{18}H_{24}O_3$ (HREIMS). The IR spectrum exhibited an OH absorption at 3417 $cm^{-1}$. In the $^1H$ NMR (Table 1) spectrum, an aromatic proton signal at $\delta$ 6.95 and an aromatic methyl at $\delta$ 2.62, as found in **3**, were missing in **4** and the signals of $-CH(CH_3)CH_2-$ were

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**Chart 1**

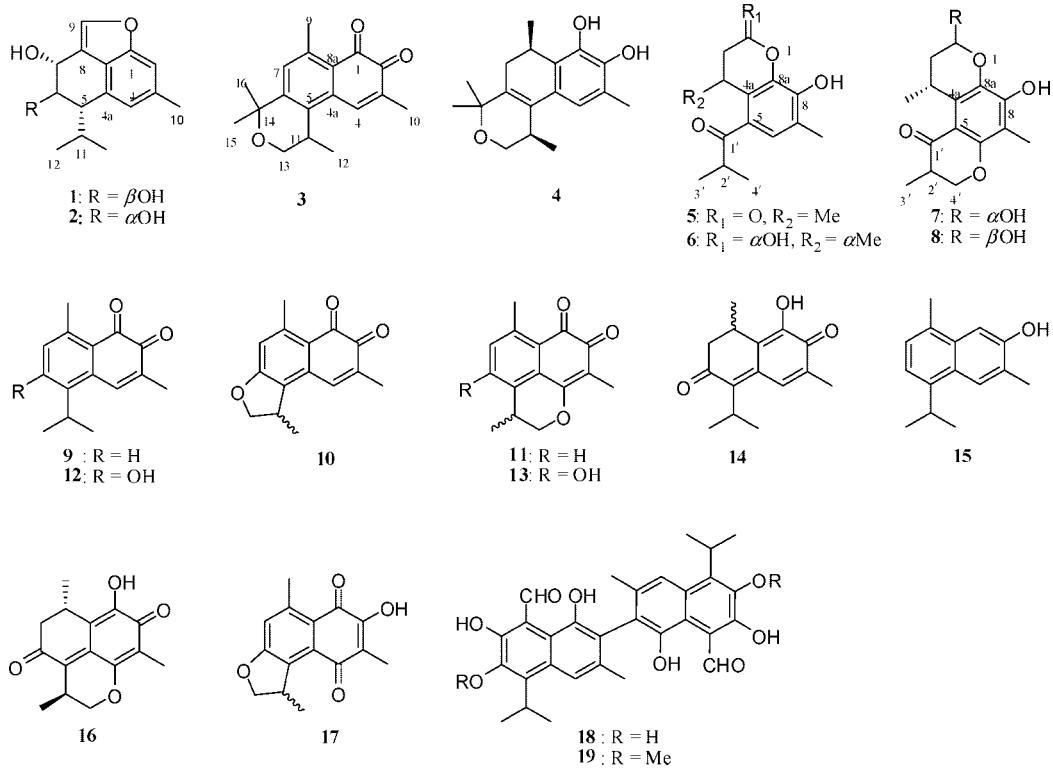


**Table 1.** $^{1}$H and $^{13}$C NMR Data for Compounds **1**−**4** (300 MHz for $^{1}$H NMR and 75 MHz for $^{13}$C NMR) in $CDCl_3$

| | **1** | | **2** | | **3** | | **4** | |
|---|---|---|---|---|---|---|---|---|
| position | $\delta_H$ (mult., *J* in Hz) | $\delta_C$ | $\delta_H$ (mult., *J* in Hz) | $\delta_C$ | $\delta_H$ (mult., *J* in Hz) | $\delta_C$ | $\delta_H$ (mult., *J* in Hz) | $\delta_C$ |
| 1 | | 153.5 | | 153.6 | | 181.7 | | 140.3 |
| 2 | 7.10 (br s) | 109.3 | 7.14 (br s) | 109.8 | | 181.6 | | 140.9 |
| 3 | | 135.8 | | 135.7 | | 135.8 | | 121.0 |
| 4 | 7.02 (br s) | 121.6 | 6.91 (br s) | 124.5 | 7.52 (d, 1.2) | 137.3 | 6.65 (s) | 117.0 |
| 4a | | 131.4 | | 129.8 | | 128.4 | | 125.0 |
| 5 | 3.02 (dd, 7.8, 3.9, $H_\beta$) | 49.8 | 2.90 (dd, 8.7, 3.3, $H_\beta$) | 53.5 | | 135.8 | | 128.7 |
| 6 | 4.01 (dd, 7.8, 7.8, $H_\alpha$) | 75.7 | 4.38 (dd, 3.3, 3.3, $H_\beta$) | 73.4 | | 150.1 | | 132.2 |
| 7 | 4.90 (dd, 7.8, 0.9, $H_\beta$) | 70.5 | 5.08 (m, $H_\beta$) | 65.6 | 6.95 (s) | 131.2 | 2.00 (d, 15.3, $H_\beta$)<br>2.36 (dd, 15.3, 5.1, $H_\alpha$) | 31.0 |
| 8 | | 118.7 | | 118.2 | | 142.6 | 3.19 (br dq, 6.9, 6.9, $H_\alpha$) | 25.2 |
| 8a | | 123.7 | | 123.4 | | 133.1 | | 125.2 |
| 9 | 7.50 (d, 0.9) | 138.8 | 7.57 (d,1.5) | 140.9 | 2.62 (s) | 23.0 | 1.04 (d, 6.9) | 17.9 |
| 10 | 2.48 (s) | 22.4 | 2.48 (s) | 22.2 | 2.09 (d, 1.2) | 16.0 | 2.25 (s) | 15.8 |
| 11 | 2.58 (m) | 27.8 | 1.63 (m) | 31.0 | 3.01 (brq, 6.9, $H_\alpha$) | 29.9 | 2.68 (m, $H_\alpha$) | 28.4 |
| 12 | 1.16 (d, 7.2)[a] | 20.0[b] | 1.12 (d, 6.6)[a] | 21.3[b] | 1.40 (d, 6.9) | 21.2 | 1.14 (d, 6.9) | 17.6 |
| 13 | 1.18 (d, 7.2)[a] | 20.8[b] | 0.94 (d, 6.6)[a] | 21.6[b] | 3.97 (dd, 11.7, 2.4, $H_\alpha$)<br>3.79 (dd, 11.7, 1.2, $H_\beta$) | 64.9 | 3.90 (dd, 11.1, 3.0, $H_\alpha$)<br>3.66 (dd, 11.1, 2.4, $H_\beta$) | 65.7 |
| 14 | | | | | | 74.9 | | 75.0 |
| 15 | | | | | 1.53 (s) | 31.3 | 1.26 (s) | 23.6 |
| 16 | | | | | 1.57 (s) | 27.8 | 1.41 (s) | 27.6 |

[a] May be interchangeable. [b] May be interchangeable.

instead observed at $\delta$ 1.04 (3H, d, $J$ = 6.9 Hz, H-9), 3.19 (1H, br dq, $J$ = 6.9, 6.9 Hz, H-8), 2.00 (1H, d, $J$ = 15.3 Hz, H-7), and 2.36 (1H, dd, $J$ = 15.3, 5.1 Hz, H-7). The assignments were confirmed by COSY cross-peaks and HMBC correlations of $H_2$-7 to C-5 ($\delta$ 128.7), C-6 ($\delta$ 132.2), and C-9 ($\delta$ 17.9) and of $H_3$-9 to C-7 ($\delta$ 31.0) and C-8a ($\delta$ 125.2). In addition, the replacement of two carbonyl carbons of the quinone ring at $\delta$ 181.7 (C-1) and 181.6 (C-2) in **3** with oxygenated aromatic carbons ($\delta$ 140.3 and $\delta$ 140.9) in **4** indicated that **4** was a reduced form of **3**. The relative configurations of H-8 and H-11 were elucidated by NOESY experiments as shown in Figure 1, which indicated that Me-9 and Me-12 were on the same side of the molecule. Thus, compound **4** was identified and named populene D.

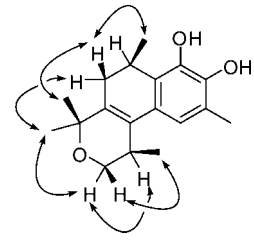

**Figure 1.** Populene D (**4**) with selected NOESY correlations.

Compound **5** had the molecular formula $C_{15}H_{18}O_4$ (HREIMS). The IR spectrum exhibited absorptions characteristic of carbonyl groups at 1776 (lactone carbonyl) and 1675 cm$^{-1}$ (conjugated

**Table 2.** $^1$H and $^{13}$C NMR Data for Compounds **5**−**8** in $CDCl_3$

| | **5**[c] | | **6**[c] | | **7**[c] | | **8**[d] | |
|---|---|---|---|---|---|---|---|---|
| position | $\delta_H$ (mult., *J* in Hz) | $\delta_C$ | $\delta_H$ (mult., *J* in Hz) | $\delta_C$ | $\delta_H$ (mult., *J* in Hz) | $\delta_C$ | $\delta_H$ (mult., *J* in Hz) | $\delta_C$ |
| 2 | | 167.4 | 5.65 (dd, 9.0, 3.0, $H_\beta$) | 92.6 | 5.56 (dd, 9.9, 2.7, $H_\beta$) | 92.2 | 5.81 (dd, 7.5, 4,5, $H_\alpha$) | 95.9 |
| 3 | 2.72 (d, 3.6) | 36.3 | 1.87 (ddd, 13.5, 9.0, 5.1, $H_\alpha$)<br>2.07 (td, 13.5, 3.0, $H_\beta$) | 36.6 | 1.84 (ddd, 13.5, 9.9, 5.4, $H_\alpha$)<br>2.04 (ddd, 13.5, 13.5, 2.7, $H_\beta$) | 36.8 | 2.00 (m) | 36.1 |
| 4 | 3.88 (tq, 7.2, 3.6) | 27.5 | 3.84 (m, $H_\beta$) | 26.2 | 4.09 (m, $H_\beta$) | 27.2 | 4.10 (m) | 27.1 |
| 4a | | 127.9 | | 126.3 | | 125.1 | | 127.2 |
| 5 | | 126.2 | | 127.1 | | 109.4 | | 111.4 |
| 6 | 7.40 (s) | 127.8 | 7.18 (s) | 125.0 | | 157.7 | | 158.7 |
| 7 | | 123.5 | | 121.0 | | 110.4 | | 110.6 |
| 8 | | 145.4 | | 146.2 | | 149.3 | | 149.9 |
| 8a | | 139.2 | | 140.0 | | 134.6 | | 134.9 |
| 1′ | | 205.8 | | 207.1 | | 195.3 | | 196.1 |
| 2′ | 3.47 (sept, 6.9) | 37.2 | 3.45 (sept, 6.9) | 37.4 | 2.75 (dq, 6.9, 5.1) | 41.2 | 2.75 (m) | 42.1 |
| 3′ | 1.21 (d, 6.9)[a] | 19.0[b] | 1.17 (d, 6.9)[a] | 19.1[b] | 1.16 (d, 6.9) | 11.0 | 1.17 (d, 6.5) | 11.8 |
| 4′ | 1.14 (d, 6.9)[a] | 19.4[b] | 1.15 (d, 6.9)[a] | 19.6[b] | 4.03 (dd, 11.1, 11.1)<br>4.43 (dd, 11.1, 5.1) | 71.6 | 4.05 (dd, 11.5, 11.5)<br>4.45 (dd, 11.5, 5.5) | 72.6 |
| 4-Me | 1.31 (d, 7.2) | 20.2 | 1.25 (d, 6.9) | 22.3 | 1.28 (d, 6.9) | 22.4 | 1.32 (d, 7.0) | 23.0 |
| 7-Me | 2.30 (s) | 15.5 | 2.23 (s) | 15.3 | 2.09 (s) | 8.1 | 2.11 (s) | 9.0 |

[a] May be interchangeable. [b] May be interchangeable. [c] 300 MHz for $^1$H NMR and 75 MHz for $^{13}$C NMR. [d] 500 MHz for $^1$H NMR and 125 MHz for $^{13}$C NMR.

carbonyl). The $^{13}$C NMR spectrum (Table 2) showed 15 resonances, which corresponded by DEPT analysis to three methines (one $sp^2$), one methylene, four methyls, and seven $sp^2$ quaternary carbons including two carbonyl carbons ($\delta_C$ 167.4 and 205.8). The $^1$H NMR (Table 2) and COSY spectra allowed assignment of signals to a dihydrocoumarin moiety[19,20] at $\delta$ 1.31 (3H, d, $J$ = 7.2 Hz, 4-Me), 2.72 (2H, d, $J$ = 3.6 Hz, $H_2$-3), 3.88 (1H, tq, $J$ = 7.2, 3.6 Hz, H-4), and 7.40 (1H, s, H-6). This moiety was also supported by the $^3J$ HMBC correlations between the methine proton H-4 and aromatic carbons C-5 ($\delta$ 126.2) and C-8a ($\delta$ 139.2), and a lactone carbonyl ($\delta$ 167.4). Signals of a 2-methyl-1-oxopropyl unit [$\delta$ 3.47 (1H, sept, $J$ = 6.9, H-2′), 1.21 (3H, d, $J$ = 6.9 Hz, H-3′), and 1.14, (3H, d, $J$ = 6.9, H-4′)] were also observed in the $^1$H NMR spectrum, whose HMBC correlation between an aromatic proton H-6 ($\delta$ 7.40) and C-1′ ($\delta$ 205.8) supported its connection at C-5 of the dihydrocoumarin moiety. An aromatic methyl signal at $\delta$ 2.30 was attributed to 7-Me due to its HMBC correlation with C-6 ($\delta$ 127.8), C-7 ($\delta$ 123.5), and C-8 ($\delta$ 145.4). Additionally, a downfield chemical shift of C-8 to $\delta$ 145.4 indicated its connection to an OH group. Compound **5** was named populene E.

Compound **6** was obtained as a yellow gum ($C_{15}H_{20}O_4$ on the basis of HREIMS). The UV and IR spectra were similar to those of **5**, but with one carbonyl absorption at 1668 $cm^{-1}$. The NMR (Table 2) data were comparable to those of **5**, except for the replacement of a lactone carbonyl ($\delta$ 167.4) in **5** with a hemiacetal proton signal of H-2 at $\delta_H$ 5.65 (dd, $J$ = 9.0, 3.0 Hz; $\delta_C$ 92.6) in **6**. The large coupling constant (13.5 Hz) was characteristic of geminal methylene protons, H-3$\beta$ (2.07, td, $J$ = 13.5, 3.0 Hz) and H-3$\alpha$ (1.87,ddd, $J$ = 13.5, 9.0, 5.1 Hz), while the vicinal coupling constants of 9.0 and 5.1 Hz indicated pseudo-*trans*-diaxial coupling of H-3$\alpha$ with H-2 and H-4, respectively. This was also in agreement with the multiplicity of H-3$\beta$ observed as a triplet of doublets with a large ($J_{gem}$ = 13.5 Hz) and a small ($J_{ax\text{-}eq}$ = 3.0 Hz) coupling constant, justifying its *syn* relationship to H-2 and H-4. Compound **6** was thus identified and named populene F.

Compound **7** was obtained as a yellow gum ($C_{15}H_{18}O_5$). The $^1$H and $^{13}$C NMR spectra (Table 2) were similar to those of **6** except that an aromatic proton (H-6) present in **6** was absent and a methyl signal (Me-4′) was replaced by oxymethylene protons resonating at $\delta$ 4.43 (1H, dd, $J$ = 11.1, 5.1 Hz, H-4′) and 4.03 (1H, dd, $J$ = 11.1, 11.1 Hz, H-4′) in **7**. A $^3J$ HMBC correlation between oxymethylene protons ($H_2$-4′) with C-6 ($\delta$ 157.7) of an aromatic moiety established their fusion by an ether linkage at C-6. The small coupling constant ($J_{2',4'ax}$ = 5.1 Hz) indicated that H-2′ was equatorially oriented. Thus, compound **7** was named populene G.

Compound **8** had the molecular formula $C_{15}H_{18}O_5$. The mass and NMR spectra of **7** and **8** indicated that they were diastereomers. The main spectroscopic differences were the downfield shift of H-2 in **8** at $\delta$ 5.81 and the smaller coupling constants (dd, $J$ = 7.5, 4.5 Hz) as compared to those of **7** at $\delta$ 5.56 (dd, $J$ = 9.9, 2.7 Hz). The coupling constant $J_{2-3}$ of 7.5 and 4.5 Hz indicated $J_{eq\text{-}ax}$ and $J_{eq\text{-}eq}$, therefore suggesting an $\alpha$-orientation of H-2. Thus, compound **8** was named populene H.

All of the isolated compounds except for **1**, **2**, **5**, **8**, **9**, and **12**, for which insufficient materials were available, were evaluated for cytotoxicity against four human cancer cell lines: breast cancer (MCF-7), cervical cancer (HeLa), colon cancer (HT-29), and oral cavity cancer (KB). They were also tested for antibacterial activity against both Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative (*Enterococcus faecalis*, *Salmonella typhi*, *Shigella sonei*, and *Pseudomonas aeruginosa*) bacteria. The results are summarized in Table 3. (+)-Gossypol (**18**) exhibited potent cytotoxic activity against HeLa and KB cell lines, with $IC_{50}$ values of 0.08 and 0.04 $\mu$g/mL, respectively. Mansonone E (**11**) showed good activity against all four cancer cell lines, especially MCF-7 ($IC_{50}$ 0.05 $\mu$g/mL). Populene D (**4**) and mansonone D (**10**) possessed strong inhibitory activity against HeLa and MCF-7, respectively, whereas populene C (**3**) exhibited moderate inhibitory activity against all four cell lines. Antibacterial activity against *B. subtilis* was found for 7-hydroxycadalene (**15**). (+)-6,6′-Methoxygossypol (**19**) was weakly active against *E. faecalis*, *B.subtilis*, and *S. aureus*, whereas (+)-gossypol (**18**) exhibited moderate activity against *B. subtilis* and *S. aureus*. None of the compounds were active against *S. typhi*, *S. sonei*, or *P. aeruginosa*. Compounds **6**, **7**, **13**, and **17** showed no cytotoxic or antibacterial activity.

## Experimental Section

**General Experimental Procedures.** Melting points were determined on an Electrothermal 9100 melting point apparatus and were uncorrected. Optical rotation was measured in $CHCl_3$ on a JASCO P-1020 polarimeter. UV spectra were measured with a SPECORD S100 spectrophotometer (Analytikjena). The IR spectra were measured with a FTS 165 FT-IR Perkin-Elmer spectrophotometer. The $^1$H and $^{13}$C NMR spectra were recorded in $CDCl_3$ using Bruker Avance 300 and 500 MHz spectrometers. The EIMS and HREIMS mass spectra were obtained from a Micromass LCT mass spectrometer. Quick column chromatography (QCC) was carried out on silica gel 60 $F_{254}$ (Merck). Precoated plates of silica gel 60 $GF_{254}$ were used for analytical purposes.

**Material.** Fresh stems of *T. populnea* were collected from Suratthani Province in the Southern part of Thailand in 2005. The plant was

**Table 3.** Cytotoxic and Antibacterial Activities of Compounds Isolated from *T. populnea*

| | cytotoxicity against human cancer cell lines, $IC_{50}$ (μg/mL) | | | | antibacterial activity, MIC (μg/mL) | | |
|---|---|---|---|---|---|---|---|
| compound | MCF-7 | HeLa | HT-29 | KB | *B. subtilis* | *S. aureus* | *E. faecalis* |
| **3** | 2.35 | 3.40 | 2.90 | 3.00 | 4.69 | –[b] | – |
| **4** | 1.85 | 0.95 | 2.37 | 3.10 | 4.69 | – | – |
| **10** | 0.80 | 2.80 | >5 | 4.90 | 2.34 | – | – |
| **11** | 0.05 | 0.55 | 0.18 | 0.40 | 4.69 | – | – |
| **14** | >5 | >5 | >5 | >5 | – | – | – |
| **15** | >5 | >5 | >5 | >5 | 0.59 | – | – |
| **16** | >5 | >5 | >5 | >5 | – | – | – |
| **18** | NT[a] | 0.08 | >5 | 0.04 | 1.17 | 1.17 | – |
| **19** | 4.00 | >5 | 3.00 | >5 | 2.34 | 4.69 | 1.17 |

[a] NT = not tested. [b] Inactive (>10 μg/mL).

identified by Prof. Puangpen Sirirugsa, and a voucher specimen (No. SB 01-001) has been deposited at the Herbarium of the Department of Biology, Prince of Songkla University (PSU).

**Extraction and Isolation of Compounds from the Dark Heartwood of *T. populnea*.** The air-dried heartwood of *T. populnea* (2.10 kg) was extracted with $CH_2Cl_2$ over a period of 5 days at room temperature. Evaporation of the solvent under reduced pressure furnished a dark residue (37.5 g). This was subjected to QCC on silica gel, eluting with $CH_2Cl_2$, and separated into eight fractions (A−H). Fraction A was purified by QCC using a gradient of hexane−acetone to afford nine fractions ($A_1$−$A_9$). Fractions $A_2$ and $A_3$ were combined and purified by QCC using a gradient of acetone−hexane as a mobile phase to give **15** (10.2 mg), **17** (8.3 mg), and **9** (2.5 mg), respectively. Fractions $A_5$ and $A_6$ were combined and then separated by QCC with a gradient system of acetone−hexane to afford **5** (2.0 mg) and **12** (2.0 mg). Fractions $A_7$ and $A_8$ were separately purified by QCC using a gradient of $CH_2Cl_2$−hexane to yield **19** (4.0 mg) from $A_7$ and **14** (4.5 mg), **11** (18.1 mg), and **18** (3.3 mg) from $A_8$. Fraction F was separated by QCC with a gradient system of increasing $CH_2Cl_2$ in hexane to afford nine fractions ($F_1$−$F_9$). Fraction $F_4$ was further purified by QCC using a gradient of $CH_2Cl_2$−hexane to give **3** (10.0 mg) and **4** (14.9 mg). Fraction $F_6$ was subjected to QCC using 20% acetone in hexane to afford four fractions ($F_{6A}$−$F_{6D}$). Fraction $F_{6B}$ was further separated by QCC with a solvent system of 2% acetone−$CHCl_3$ to afford **6** (12.6 mg). Fraction $F_{6C}$, upon standing overnight at room temperature, gave a yellow solid of **8** (4.2 mg), and the mother liquor gave **7** (4.1 mg). Fraction G was purified by QCC with a gradient of acetone−$CH_2Cl_2$ to give five fractions ($G_A$−$G_E$). Fraction $G_A$ was subjected to precoated TLC using 50% $CH_2Cl_2$−hexane (4 runs) to give **16** (5.1 mg). Fraction $G_C$ gave **10** (93.0 mg). Fraction H, upon standing overnight at room temperature, gave red-brown crystals of **13** (30.5 mg).

**Extraction and Isolation of Compounds from the Wood of *T. populnea*.** The air-dried wood of *T. populnea* (1.40 kg) was extracted with $CH_2Cl_2$ over a period of 5 days at room temperature. Evaporation of the solvent under reduced pressure furnished a dark green residue (10.2 g). This was subjected to QCC on silica gel, eluted with a gradient of hexane−acetone to give six fractions (A−F). Fraction C was then purified by QCC using a gradient of hexane−acetone to afford **18** (22.6 mg). Fraction D, upon standing overnight at room temperature, gave **19** (20.3 mg). Fraction E was separated by QCC with a gradient system of increasing polarity (acetone−hexane) to afford five fractions ($E_1$−$E_5$). Fraction $E_2$ was subjected to precoated plates using 50% $CH_2Cl_2$−hexane as a mobile phase (4 runs) to give **11** (1.6 mg). Fraction $E_3$ was subjected to precoated plates using 3% MeOH−$CH_2Cl_2$ as a mobile phase (4 runs) to give **1** (2.3 mg) and **2** (2.1 mg).

**Populene A (1):** yellow gum; $[\alpha]^{25}_D$ +57.9 (*c* 0.54, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log ε) 216 (4.22), 251 (3.98), 259 (3.91), 279 (3.44), 289 (3.40) nm; IR (neat) $\nu_{max}$ 3365, 2959, 2870, 1617, 1591, 758 $cm^{-1}$; NMR data see Table 1; EIMS *m/z* 246 $[M]^+$ (8), 211 (18), 185 (33), 169 (25), 72 (100), 69 (47); HREIMS *m/z* 246.1262 (calcd for $C_{15}H_{18}O_3$, 246.1256).

**Populene B (2):** yellow gum; $[\alpha]^{25}_D$ −63.6 (*c* 0.37, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log ε) 213 (4.15), 251 (3.85), 259 (3.80), 278 (3.32), 290 (3.29) nm; IR (neat) $\nu_{max}$ 3387, 2959, 2871, 1716, 1524, 754 $cm^{-1}$; NMR data see Table 1; EIMS *m/z* 246 $[M]^+$ (50), 199 (31), 185 (100), 157 (23), 129 (46); HREIMS *m/z* 246.1255 (calcd for $C_{15}H_{18}O_3$, 246.1256).

**Populene C (3):** orange solid; mp 168−170 °C; $[\alpha]^{25}_D$ −46.0 (*c* 0.27, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log ε) 213 (4.18), 242 (3.79), 259 (3.98), 380 (3.03) nm; IR (neat) $\nu_{max}$ 2974, 2930, 2871, 1757, 1698, 1657 $cm^{-1}$; NMR data see Table 1; EIMS *m/z*, 286.1556 $[M + 2]^+$ (17), 271 (53), 241 (72), 85 (66), 83 (100); HREIMS *m/z* 286.1556 $[M+2]^+$ (calcd for $C_{18}H_{24}O_3$, 284.1412).

**Populene D (4):** brown gum; $[\alpha]^{25}_D$ −21.9 (*c* 0.75, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log ε) 219 (4.10), 264 (3.92), 277sh (3.81), 366 (2.86) nm; IR (neat) $\nu_{max}$ 3417, 2967, 2930, 2863, 1653, 754 $cm^{-1}$; NMR data see Table 1; EIMS *m/z* 288 $[M]^+$ (15), 274 (21), 241 (20), 273 (100); HREIMS *m/z* 288.1736 (calcd for $C_{18}H_{24}O_3$, 288.1725).

**Populene E (5):** yellow-brown gum: $[\alpha]^{25}_D$ +30.1 (*c* 0.58, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log ε) 228 (4.11), 273 (3.86) nm; IR (neat) $\nu_{max}$ 3410, 2970, 2925, 2873, 1776, 1675, 1616 $cm^{-1}$; NMR data see Table 2; EIMS *m/z* 262 $[M]^+$ (31), 220 (34), 191 (43), 219 (100); HREIMS *m/z* 262.1210 (calcd for $C_{15}H_{18}O_4$, 262.1205).

**Populene F (6):** yellow gum; $[\alpha]^{25}_D$ +7.5 (*c* 0.23, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log ε) 219 (4.23), 232 (4.14), 281 (3.00) nm; IR (neat) $\nu_{max}$ 3417, 2967, 2930, 2871, 1668, 1576 $cm^{-1}$; NMR data see Table 2; EIMS *m/z* 264 $[M]^+$ (27), 221 (100), 203 (22), 193 (26), 179 (44), 177 (25), 151 (20); HREIMS *m/z* 264.1353 (calcd for $C_{15}H_{20}O_4$, 264.1362).

**Populene G (7):** yellow gum; $[\alpha]^{25}_D$ +62.7 (*c* 0.07, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log ε) 214 (4.09), 235 (3.98), 286 (3.95), 339 (3.66) nm; IR (neat) $\nu_{max}$ 3424, 2959, 2930, 2871, 1661, 1591, 1429 $cm^{-1}$; NMR data see Table 2; EIMS *m/z* 278 $[M]^+$ (98), 249 (27), 239 (100), 208 (36), 192 (35); HREIMS *m/z* 278.1196 (calcd for $C_{15}H_{18}O_5$, 278.1154).

**Populene H (8):** yellow gum; $[\alpha]^{25}_D$ +43.7 (*c* 0.04, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log ε) 214 (4.06), 237 (3.95), 286 (3.96), 339 (3.60) nm; IR (neat) $\nu_{max}$ 3417, 2967, 2930, 2871, 1661, 1587 $cm^{-1}$; NMR data see Table 2; EIMS *m/z* 278 $[M]^+$ (54), 234 (56), 208 (25), 192 (24), 72 (100); HREIMS *m/z* 278.1159 (calcd for $C_{15}H_{18}O_5$, 278.1154).

**Antimicrobial Assay.** The compounds isolated from *T. populnea* were tested against the microorganisms *Bacillus subtilis* (obtained from Department of Industrial Biotechnology, PSU), *Staphylococcus aureus* (TISTR517) (obtained from Microbial Resources Center (MIRCEN), Bangkok, Thailand), *Pseudomonas aeruginosa, Enterococcus faecalis*, *Shigella sonei*, and *Salmonella typhi*. The last four microorganisms were obtained from the Department of Pharmacognosy and Botany, PSU. The antibacterial assay employed was the same as described in Boonsri et al.[10] Vancomycin, which was used as a standard, showed an antibacterial activity of 0.078 μg/mL.

**Cytotoxic Assay.** The procedure for the cytotoxic assay was performed by the sulforhodamine B (SRB) assay as described by Skehan et al.[21] In this study, four cancer cell lines obtained from the National Cancer Institute, Bangkok, Thailand, were used: MCF-7 (breast adenocarcinoma), KB (human oral cancer), HeLa (human cervical cancer), and HT-29 (colon cancer). Camptothecin, which was used as a standard, showed cytotoxic activity in the range 0.2−2.0 μg/mL.

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