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# TRADITIONAL MEDICINAL PLANTS OF THAILAND, VIII. ISOFLAVONOIDS OF *DALBERGIA CANDENATENSIS*[1]

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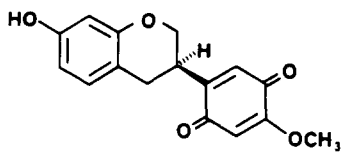
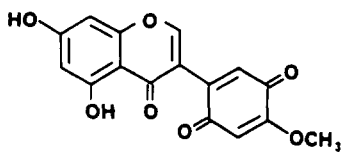
ABSTRACT.—Five isoflavonoids, including the new isoflavone quinone, 5-hydroxybowdichione [**2**], were isolated from the heartwood of *Dalbergia candenatensis* through bioactivity-directed fractionation.

Continuing our studies on the traditional medicinal plants of Thailand (1), we have investigated *Dalbergia candenatensis* Prain (Leguminosae), a tree indigenous to eastern Asia. The heartwood has a deep red color and is used in Thailand as a red dyestuff. There have been no previous phytochemical studies on this species. In a preliminary biological evaluation, the $CHCl_3$ and MeOH extracts were found to display antibacterial and antifungal properties. The same extracts also showed cytotoxic activity against the P-388 lymphocytic leukemia test system in vitro (2).

The isolation of the antibacterial and antifungal components was monitored by direct bioautographic assay on tlc (3,4). Four of the isolated compounds were identified as (±)-mucronulatol (5,6), (*R*)-(−)-claussequinone [**1**] (7), formononetin (5), and (*R*)-(−)-vestitol (7,8),. The new isoflavone quinone **2** was characterized in the following manner.




**1**




**2**

The bathochromic shift in band II of the uv spectrum, on the addition of $AlCl_3$/HCl and NaOAc, indicated the presence of a hydroxy group *peri* to a carbonyl group and of a second acidic hydroxy group. In the ir spectrum a complex absorption pattern was observed in the region 1650-1680 $cm^{-1}$ suggesting the presence of more than one α,β-unsaturated ketone moiety. In the mass spectrum obtained at 20 eV, compound **2** showed a fragmentation pattern typical for methoxybenzoquinones (9).

An intense molecular ion was observed at *m/z* 314. Elimination of a methyl group and subsequent loss of CO resulted in ions at *m/z* 299 and 271, respectively. The ion at *m/z* 243 arose from the loss of a methyl group and two molecules of CO, and this ion decomposed by further elimination of CO leading to a $M^+$-99 species at *m/z* 215. A fragment ion at *m/z* 203 was due to the rupture of the C-1′-C-2′ and C-5′-C-6′ bonds, and an intense peak, characteristic of methoxybenzoquinones, appeared at *m/z* 69 ($C_3HO_2^+$). RDA fragmentation of the molecule and subsequent elimination of CO led to the ions at *m/z* 152 and *m/z* 124.

In the relatively simple $^1$H-nmr spectrum a singlet at 8.36 ppm was assigned to H-2 of an isoflavone with the resonances for the hydroxyl groups at C-5 and C-7 appearing at 12.46 and 11.06 ppm, respectively. Two one-proton singlets for H-3′ and H-6′ were observed at 6.26 and 7.05 ppm, respectively. Assignment of the $^{13}$C-nmr spec-

trum of **2** was achieved through comparison with literature data (10,11), with the data for claussequinone [**1**], and by the attached proton test (APT) spectrum. Based on this spectroscopic information compound **2** has been identified as 5-hydroxybowdichione, the second isoflavone quinone to be described (12).

The in vitro antifungal and antibacterial activities of the isolated isoflavonoids are listed in Table 1 using tetracycline and and nystatin as reference compounds. Minimum inhibitory concentrations (MIC) were determined through a microdilution assay (13). The three major isoflavonoids, mucronulatol, formononetin, and vestitol, exhibited only marginal or no antibacterial activity at the concentrations tested. The reason why these compounds were detected through bioautography was their high concentration in the extracts. Claussequinone [**1**], however, showed good antibacterial activity with MIC values ranging from 3 to 6 μg/ml. 5-Hydroxybowdichione [**2**], which had previously shown good activity in the bioautographic assay, was only weakly active in the microdilution test. We attribute this diminished activity to the instability of this compound in solution.

TABLE 1. Antimicrobial Activities of Isoflavonoids and Reference Compounds

| Microorganism | Compound | | | | | | |
|---|---|---|---|---|---|---|---|
| | mucronulatol | claussequinone [1] | 5-hydroxybowdichione [2] | formononetin | vestitol | tetracycline | nystatin |
| *Staphylococcus aureus* ATCC 25923 | >100[a] | 6 | >100 | >100 | >100 | 0.6 | |
| *Bacillus subtilis* ATCC 6633 | >100 | 3 | 100 | >100 | 50 | 2.5 | |
| *Escherichia coli* ATCC 25922 | 50 | 6 | 50 | 100 | 50 | 1.2 | |
| *Candida albicans* ATCC 10259 | >100 | 50 | >100 | >100 | >100 | | 1.5 |
| *Penicillium expansum* ATCC 7861 | >100 | >100 | >100 | 50 | >100 | | 6.0 |
| *Aspergillus niger* ATCC 6275 | >100 | 100 | >100 | 100 | >100 | | 6.0 |
| *Trichophyton mentagrophytes* ATCC 9533 | 25 | 25 | 100 | >100 | 25 | | 3.0 |

[a]Minimum inhibitory concentration (μg/ml).

Although none of the five isoflavonoids displayed significant activity against *Candida albicans, Aspergillus niger,* and *Penicillium expansum*, mucronulatol, claussequinone [**1**], and vestitol, displayed an MIC of 25 μg/ml against *Trichophyton mentagrophytes*, a dermatophyte causing ringworm and athelete's foot.

Claussequinone [**1**] displayed an $ED_{50}$ 0.5 μg/ml against the P-388 lymphocytic leukemia test system in vitro. Quinone **2** was inactive in this test system, again possibly due to its instability in solution.

## EXPERIMENTAL

GENERAL PROCEDURES.—Melting points were determined on Kofler hot-plate apparatus and are uncorrected. Specific rotations were measured on a Perkin-Elmer 241 polarimeter. The uv spectra were obtained with a Beckman DU-7 spectrophotometer.

Ir spectra were measured on a Nicolet MX-1 FT-IR spectrophotometer. $^1$H-nmr and $^{13}$C-nmr spectra were recorded with a Nicolet NT 1280 spectrometer operating at 360 and 90.8 MHz, respectively, or on a Varian XL300 instrument at 300 MHz and 75.44 MHz, respectively. TMS was used as an internal standard. Low-resolution mass spectra were obtained with a Varian MAT-112S mass spectrometer.

Tlc was performed on Si gel coated Al sheets (Merck, Darmstadt, W. Germany); detection was performed at 254 and 366 nm, and after spraying with anisaldehyde/$H_2SO_4$.

PLANT MATERIAL.—The dried heartwood of *D. candenatensis* was purchased in 1984 in Bangkok, Thailand, in a local market. The plant material was authenticated by comparison with voucher specimens deposited in the Botany Section, Technical Division, Department of Agriculture and Cooperatives, Bangkok, Thailand.

BIOASSAYS.—Cytotoxic activity was determined using cultured P-388 cells, as described previously (2). Bioautography for antifungal and antibacterial compounds were performed according to (3) and (4), respectively. MICs of the pure compounds were determined in a microdilution assay according to Swenson *et al.* (13), using Mueller-Hinton broth for the bacteria and Sabourand-Dextrose broth (Difco, Detroit, MI) for yeast and fungi. The microbial activity was measured after 16 h incubation at 37 ° for the bacteria and yeast and after a 3 day incubation at 27° for the fungi. The compounds were dissolved in DMSO and diluted such that the final concentration of DMSO was no more than 1%.

MICROORGANISMS.—Strains were purchased from the American Type Culture Collection (Rockville, MD). The bacteria were cultured in nutrient broth (BBL, Lockeysville, MD); the yeast and fungi were cultured in yeast-malt or Sabourand-Dextrose broth (BBL).

EXTRACTION AND PRELIMINARY BIOLOGICAL EVALUATION.—The powdered plant material (430 g) was extracted at room temperature successively with light petroleum ether, $CHCl_3$, and MeOH. The $CHCl_3$ and MeOH extracts showed an $ED_{50}$ of 2.5 μg/ml and 27 μg/ml, respectively, against the P-388 test system in vitro. Neither extract exhibited significant activity against the KB cell culture system. The presence of antifungal and antibacterial compounds in both extracts was detected by bioautography on Si gel ($CHCl_3$-MeOH, 95:5).

ISOLATION OF ISOFLAVONOIDS.—The pigments of the MeOH extract (100 g) were removed by chromatography over Si gel eluting with EtOAc-MeOH-$H_2O$ (100:6:3). The active fraction was further separated on Si gel eluting with $CHCl_3$-MeOH (95:5) into five fractions (A-E). Recrystallization of fraction B yielded (*R,S*)-(+)-mucronulatol as colorless cubes (300 mg); mp 226-228° ($CHCl_3$/MeOH) [lit. (5) mp 224-225°]; [α]D, uv, ir, eims: see (5,6); $^1$H nmr (360 MHz, DMSO-$d_6$) δ 2.71 (1H, dd, $J$=13.0, 5.0 Hz, 4-$H_{eq}$), 2.84 (1H, dd, $J$=13.0, 11.3 Hz, 4-$H_{ax}$), 3.30 (1H, ddd, $J$=11.2, 10.3, 3.0, Hz, 3-$H_{ax}$), 3.73 (3H, s, $OCH_3$), 3.75 (3H, s, $OCH_3$), 3.90 (1H, dd, $J$=10.3, 10.3 Hz, 2-$H_{ax}$), 4.10 (1H, ddd, $J$=10.3, 5.0, 3.0 Hz, 2-$H_{eq}$), 6.19 (1H, d, $J$=2.3 Hz, 8-H), 6.29 (1H, dd, $J$=8.2, 2.4 Hz, 6-H), 6.60-6.69 (2H, ABq, 5′-H, 6′-H), 6.86 (1H, d, $J$=8.3 Hz, 5-H), 8.66 (1H, s, 3′-OH), 9.18 (1H, s, 7-OH); $^{13}$C nmr (90.8 MHz, DMSO-$d_6$) δ 30.8 (C-4), 31.3 (C-3), 55.9 (2′-$OCH_3$), 60.3 (4′-$OCH_3$), 69.7 (C-2), 102.5 (C-8), 107.5 (C-5′), 107.9 (C-6), 112.7 (C-4a), 116.2 (C-1′), 126.8 (C-6′), 130.1 (C-5), 139.2 (C-3′), 146.0 (C-2′), 147.7 (C-4′), 154.5 (C-8a), 156.5 (C-7).

Fraction C was rechromatographed over Si gel eluting with $CHCl_3$-MeOH (98:2) to afford formononetin (95 mg), mp 260-262° [lit. (5) mp 257-257.5°], (*R*)-(−)-claussequinone [**1**] (155 mg) and 5-hydroxybowdichione [**2**], (4 mg) as crystalline compounds.

(R)-(−)-*Claussequinone* [**1**].—Mp 193-196° decomp. [lit. (7) mp 189-194° decomp.]; [α]D, uv, ir, eims: see lit. (7); $^1$H nmr (360 MHz, DMSO-$d_6$) δ 2.72 (1H, dd, $J$=15.5, 9.0 Hz, 4-$H_{ax}$), 2.80 (1H, dd, $J$=15.5, 5.7 Hz, 4-$H_{eq}$), 3.21 (1H, m, 3-$H_{ax}$), 3.78 (1H, s, 4′-$OCH_3$), 3.92 (1H, dd, $J$=10.5, 8.4 Hz, 2-$H_{ax}$), 4.16 (1H, dd, $J$=10.4, 1.9 Hz, 2-$H_{eq}$), 6.17 (1H, s, 3′-H), 6.18 (1H, d, $J$=2.5 Hz, 8-H), 6.30 (1H, dd, $J$=8.3, 2.3 Hz, 6-H), 6.54 (1H, s, 6′-H), 6.86 (1H, d, $J$=8.3 Hz, H-5), 9.25 (1H, bs, 7-OH); $^{13}$C nmr (90.8 MHz, DMSO-$d_6$) δ 28.8 (C-4), 30.6 (C-3), 56.4 (4′-$OCH_3$), 67.9 (C-2), 102.5 (C-8), 107.9 (C-6), 108.4 (C-3′), 111.3 (C-4a), 130.0 (C-5), 130.4 (C-6′), 148.4 (C-1′), 154.2 (C-8a), 156.6 (C-7), 158.3 (C-4′), 181.7 (C-5′), 186.5 (C-2′).

*5-Hydroxybowdichione* [**2**].—Yellow crystals, mp 241-245° decomp. ($CHCl_3$/MeOH); uv λ max (MeOH) 318 sh, 279, 262, and 223 nm sh, (+NaOMe) 334, 292, 240 nm sh; (+NaOAc) 328, 271 nm; (+$AlCl_3$) 372, 308, 269 nm, no alteration on addition of HCl; ir ν max (KBr) 3480, 3440, 1660, 1647, 1630, 1621, 1598, 1585, 1233, 1205, 1190, 1170, 1154, 830, 825 $cm^{-1}$; $^1$H nmr (300 MHz, DMSO-$d_6$) δ 3.83 (3H, s, 4′-$OCH_3$), 6.26 (1H, s, 3′-H), 6.27 (1H, d, $J$=1.5 Hz, 8-H), 6.44 (1H, d, $J$=1.5 Hz, 6-H), 7.05 (1H, s, 6′-H), 8.36 (1H, s, 2-H), 11.06 (1H, s, 7-OH), 12.46 (1H, s, 5-OH); $^{13}$C nmr (75.44 MHz, DMSO-$d_6$) δ 56.3 (4′-$OCH_3$), 94.1 (C-8), 99.5 (C-6), 104.0 (C-4a), 107.9 (C-3′), 115.5 (C-3), 133.2 (C-6′), 137.9 (C-1′), 157.1 (C-8a), 157.5 (C-2), 158.4 (C-4′), 161.6 (C-5), 164.7 (C-7), 178.8 (C-4), 181.4 (C-5′), 185.0 (C-2′); ms (20 eV) *m/z* (rel. int.) 314 (100), 299 (17), 271 (99), 255 (3), 243 (3), 229 (6), 215 (7), 203 (14), 153 (7), 152 (6), 124 (6), 69 (37).

Fraction D on column chromatography over Si gel eluting with $CHCl_3$-MeOH (98:2) as eluent, yielded (*R*)-(−)-vestitol (420 mg) as colorless crystals, mp 157-159° ($CHCl_3$/MeOH) [lit. (7) mp 154-

157°]; $[\alpha]_D$, uv, ir, eims, see lit. (7,8); $^1$H nmr (360 MHz, DMSO-$d_6$) δ 2.71 (1H, dd, $J$=15.0, 4.5 Hz, 4-$H_{eq}$), 2.88 (1H, dd, $J$=15.0, 11.3 Hz, 4-$H_{ax}$), 3.32 (1H, ddd, $J$=11.3, 10.5, 3.0 Hz, 3-$H_{ax}$), 3.68 (3H, s, 4′-$OCH_3$), 3.91 (1H, dd, $J$=10.5, 10.5 Hz, 2-$H_{ax}$), 4.14 (1H, ddd, $J$=10.5, 4.5, 3.0 Hz, 2-$H_{eq}$), 6.18 (1H, d, $J$=1.9 Hz, 8-H), 6.28 (1H, dd, $J$=8.1, 2.0 Hz, 6-H), 6.36 (1H, dd, $J$=8.2, 2.1 Hz, 5′-H), 6.42 (1H, d, $J$=2.1 Hz, 3′-H), 6.87 (1H, d, $J$=8.2 Hz, 5-H), 6.99 (1H, d, $J$=8.4 Hz, 6′-H), 9.10 (1H, s, 7-OH), 9.53 (1H, s, 2′-OH); $^{13}$C nmr (90.8 MHz, DMSO-$d_6$) δ 29.8 (C-4), 31.1 (C-3), 54.8 (4′-$OCH_3$), 69.2 (C-2), 101.4 (C-3′), 102.5 (C-8), 104.3 (C-5′), 107.9 (C-6), 112.7 (C-4a), 119.7 (C-1′), 127.6 (C-6′), 129.9 (C-5), 154.5 (C-8a), 155.8 (C-2′) 156.5 (C-7), 158.7 (C-4′).

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