CHEMICAL CONSTITUENTS OF THE STEMS OF *DRACAENA LOUREIRI*. STRUCTURAL MODIFICATION OF RESVERATROL AND CYTOTOXICITY EVALUATION OF THE ANALOGUES

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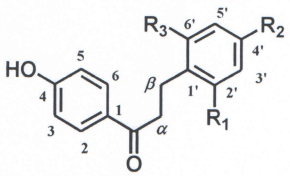
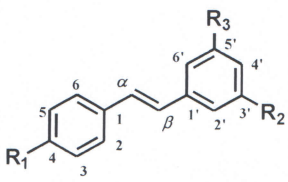
Abstract: Investigation of the MeOH extract of the stems of *Dracaena loureiri* led to the isolation of four dihydrochalcones, loureirin B (1), 2,4′-dihydroxy-4,6-dimethoxydihydrochalcone (2), loureirin C (3) and 2,4,4′-trihydroxy-6-methoxydihydrochalcone (4), and one stilbene, resveratrol (5). Resveratrol derivatives (6-15) and resveratrol dimers (16 and 17) were synthesized and identified. All natural compounds and synthetic analogues were evaluated for cytotoxicity against HeLa, HT 29 and MCF-7 cell lines.

1. Introduction

The stems of *Dracaena loureiri* have been used as a folk medicine for the treatment of antipyretic, anti-inflammatory, antibacterial and cytotoxic activities against leukemia and human carcinoma cells, estrogenic activity and pain relief [1-6]. In our study, four dihydrochalcone analogues, loureirin B (**1**), 2,4′-dihydroxy-4,6-dimethoxydihydrochalcone (**2**), loureirin C (**3**) and 2,4,4′-trihydroxy-6-methoxy dihydrochalcone (**4**), and one stilbene, resveratrol (**5**), from the MeOH extract of the stems of *D. loureiri* were isolated. In the present study, resveratrol, the major component, was subjected to structural modification. Cytotoxicity evaluations of the synthesized compounds have been undertaken.



1: R_1=R_2=R_3=OMe
2: R_1=OH, R_2=R_3=OMe
3: R_1=OMe, R_2=OH, R_3=H
4: R_1=R_2=OH, R_3=OMe



5: R_1=R_2=R_3=OH
6: R_1=OMe, R_2=R_3=OH
7: R_2=OMe, R_1=R_3=OH
8: R_1=R_2=OMe, 3_3=OH
9: R_2=R_3=OMe, R_1=OH
10: R_1=R_2=R_3=OMe
11: R_1=OAc, R_2=R_3=OH
12: R_2=OAc, R_1=R_3=OH
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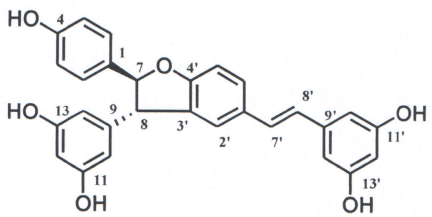
14: R_2=R_3=OAc, R_1=OH
15: R_1=R_2=R_3=OAc



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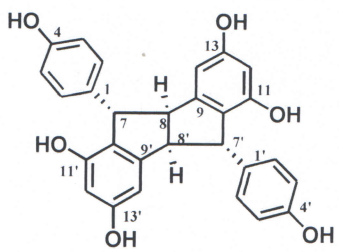

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2. Materials and Methods

2.1 General Procedure

Optical rotation was measured on a JASCO-1020 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 spectrometer. Mass spectra were obtained on a Finnigan LC-Q mass spectrometer. Column chromatography and TLC were carried out using Merck silica gel 60 (<0.063 mm) and precoated silica gel 60 F_{254} plates, respectively. Spots on TLC

were detected under UV light and by spraying with anisaldehyde-H_2SO_4 reagent followed by heating.

2.2 Plant material

The dried stems of *D. loureiri* used in this study were purchased from Tai An Chan herbal store, Bangkok, in 2010.

2.3 Isolation of 1-5

The dry stems of *D. loureiri* (10.0 kg) were milled and extracted successively with *n*-hexane, EtOAc and MeOH. The extracts were evaporated to dryness under reduced pressure at temperature 40-45 °C. The MeOH extract (1.17 kg) was subjected to column chromatography eluted with *n*-hexane, *n*-hexane-EtOAc, EtOAc, EtOAc-MeOH and MeOH with increasing amount of the more polar solvent to give 8 fractions. Fraction 4 (14.96 g) was subjected to column chromatography eluted with CH_2Cl_2:MeOH (100:2) to give 8 subfractions. Subfraction 2 was chromatographed eluting with *n*-hexane:EtOAc (80:20) to afford compound **1** (35.2 mg). Subfraction 6 was chromatographed eluting with CH_2Cl_2: MeOH (100:2) to afford compound **3** (31.2 mg). Fraction 5 (7.38 g) was subjected to column chromatography eluted with CH_2Cl_2:MeOH (150:1), *n*-hexane:EtOAc (70:30) and CH_2Cl_2:MeOH (100:2) to give compound **2** (16.1 mg). Fraction 6 (85.91 mg) was similarly chromatographed using *n*-hexane:EtOAc (60:40) and recrystallization with CH_2Cl_2 to yield compound **4** (86.0 mg). The filtrate was subjected to column chromatography eluting with CH_2Cl_2:MeOH (100:4), and CH_2Cl_2:MeOH (100:2) to give compound **5** (1.63 g).

Compound **1**: Pink needles. IR ν_{max} cm^{-1}: 3389, 2940, 2834, 1645, 1541, 1452, 1124, 822, 793. ESMS *m/z*: 317 $[M+H]^+$. The ^1H NMR data are shown in Table 1. Compound **1** was identified as loureirin B by comparison of the ^1H NMR data with those of the reported values [7].

Compound **2**: Dark pink solid. IR ν_{max} cm^{-1}: 3263, 2941, 2837, 1646, 1577, 1147, 812, 720. ESMS *m/z*: 303 $[M+H]^+$. The ^1H NMR data are shown in Table 1. Compound **2** was identified as 2,4′-dihydroxy-4,6-dimethoxydihydrochalcone by comparison of the ^1H NMR data with those of the reported values [5].

Compound **3**: Pink needles. IR ν_{max} cm^{-1}: 3302, 2938, 2843, 1654, 1595, 1452, 1147, 833, 760 830. ESMS *m/z*: 273 $[M+H]^+$. The ^1H NMR data are shown in Table 1. Compound **3** was identified as loureirin C by comparison of the ^1H NMR data with those of the reported values [7].

Compound **4**: Pale brown solid. IR ν_{max} cm^{-1}: 3398, 3027, 2949, 1655, 1574, 1472, 1174, 821, 781. ESMS *m/z*: 289 $[M+H]^+$. The ^1H NMR data are shown in Table 1. Compound **4** was identified as 2,4,4′-trihydroxy-6-methoxydihydrochalcone by comparison of the ^1H NMR data with those of the reported values [7].

Compound **5**: Pale pink powder. IR ν_{max} cm^{-1}: 3201, 3021, 1583, 1380, 1144, 827. ESMS *m/z*: 229 $[M+H]^+$. The ^1H NMR data are shown in Table 2. Compound **5** was identified as resveratrol by comparison of the ^1H NMR data with those of the reported values [4].

2.4 Synthesis of 6-17

Methylation of **5**. A solution of compound **5** (100 mg) in dry DMF (10 ml) was reacted with MeI (0.5 ml) and K_2CO_3 (100 mg). The reaction mixture was stirred at 0 °C for 1 h. Water (30 ml) was added and the solution was extracted with CH_2Cl_2 (100 ml). The organic phase was washed with water (2×20 ml) and dried over anhydrous Na_2SO_4. Removal of the solvent gave the crude methyl ether of **5** which was isolated and purified by column chromatography using CH_2Cl_2 to afford compound **6** (15.9 mg), compound **7** (15.5 mg), compound **8** (12.0 mg), compound **9** (11.9 mg), and compound **10** (29.5 mg).

Compound **6**: Yellow amorphous solid. IR ν_{max} cm^{-1}: 3434, 3019, 2943, 1586, 1433, 1163, 830. ESMS *m/z*: 242 $[M]^+$. The ^1H NMR data are shown in Table 2. Compound **6** was identified as resveratrol 4-methyl ether by comparison of the ^1H NMR data with the reported values [8].

Compound **7**: Pale orange amorphous solid. IR ν_{max} cm^{-1}: 3435, 3094, 3021, 1586, 1194, 840. ESMS *m/z*: 241 $[M-H]^-$. The ^1H NMR data are shown in Table 2. Compound **7** was identified as resveratrol 3′-methyl ether by comparison of the ^1H NMR data with the reported values [4].

Compound **8**: Pale yellow amorphous solid. IR ν_{max} cm^{-1}: 3445, 3027, 2932, 1586, 1433, 1163, 830. ESMS *m/z*: 255 $[M-H]^-$. The ^1H NMR data are shown in Table 2. Compound **8** was identified as resveratrol 4,3′-dimethyl ether by comparison of the ^1H NMR data with the reported values [8].

Compound **9**: Pale yellow amorphous solid. IR ν_{max} cm^{-1}: 3435, 3018, 2949, 1586, 1433, 1194, 840. ESMS *m/z*: 257 $[M+H]^+$. The ^1H NMR data are shown in Table 2. Compound **9** was identified as resveratrol 3′,5′-dimethyl ether by comparison of the ^1H NMR data with the reported values [4].

Compound **10**: Pale brown amorphous solid. IR ν_{max} cm^{-1}: 3005, 2927, 2836, 1589, 1456, 1150, 829. ESMS *m/z*: 271 $[M+H]^+$. The ^1H NMR data are shown in Table 2. Compound **10** was identified as resveratrol 4,3′,5′-trimethyl ether by comparison of the ^1H NMR data with the reported values [8].

Acetylation of **5**. A mixture of compound **5** (100 mg), pyridine (2 ml) and acetic anhydride (0.5 ml) was stirred at 0 °C for 1 h. Water (30 ml) was added and the solution was extracted with CH_2Cl_2 (100 ml). The organic phase was washed with water (2×20 ml) and dried over anhydrous Na_2SO_4. Removal of the solvent gave the crude acetylated analogues of **5** which was chromatographed using CH_2Cl_2 to yield s **11** (37.7 mg), **12** (17.7 mg), **13** (15.7 mg), **14** (9.2 mg), and **15** (26.4 mg).

Compound **11**: Pale brown amorphous solid. IR ν_{max} cm^{-1}: 3259, 3027, 2938, 1715, 1605, 1509, 1194, 833. ESMS *m/z*: 271 $[M+H]^+$. The ^1H NMR data are shown in Table 2. Compound **11** was identified as

resveratrol 4-acetate by comparison of the ^{1}H NMR data with the reported values [9].

Compound **12**: Pale brown amorphous solid. IR ν_{max} cm^{-1}: 3250, 3038, 2938, 1715, 1605, 1509, 1150, 834. ESMS *m/z*: 269 $[M-H]^-$. The ^{1}H NMR data are shown in Table 2. Compound **12** was identified as resveratrol 3′-acetate by comparison of the ^{1}H NMR data with the reported values [9].

Compound **13**: Orange amorphous solid. IR ν_{max} cm^{-1}: 3272, 3032, 2927, 1715, 1626, 1440, 1246, 833. ESMS *m/z*: 311 $[M-H]^-$. The ^{1}H NMR data are shown in Table 2. Compound **13** was identified as resveratrol 4,3′-diacetate by comparison of the ^{1}H NMR data with the reported values [9].

Compound **14**: Pale red amorphous solid. IR ν_{max} cm^{-1}: 3260, 3027, 2927, 1715, 1605, 1509, 1194, 833. ESMS *m/z*: 311 $[M-H]^-$. The ^{1}H NMR data are shown in Table 2. Compound **14** was identified as resveratrol 3′,5′-diacetate by comparison of the ^{1}H NMR data with the reported values [9].

Compound **15**: White powder. IR ν_{max} cm^{-1}: 3077, 3049, 2943, 1752, 1578, 1187, 1124, 861. ESMS *m/z*: 377 $[M+Na]^+$. The ^{1}H NMR data are shown in Table 2. Compound **15** was identified as resveratrol 4,3′,5′-triacetate by comparison of the ^{1}H NMR data with the reported values [9].

Oxidative coupling of **5** with $K_3Fe(CN)_6$. To the solution of **5** (100 mg) in methanol (3 ml) was added water (0.5 ml) and $K_3Fe(CN)_6$ (150 mg), and the mixture was kept stirring. After 8 h, water was added and the mixture was extracted with EtOAc (3×20 ml), and the combined organic layer was dried over anhydrous Na_2SO_4. The residue was subjected to column chromatography using CH_2Cl_2:MeOH (100:2) to yield compounds **16** (35.2 mg) and **17** (27.8 mg).

Compound **16**: Brown amorphous solid. IR ν_{max} cm^{-1}: 3272, 3016, 2915, 1597, 1509, 1125, 830. ESMS *m/z*: 453 $[M-H]^-$. ^{1}H NMR (400 MHz, CD_3COCD_3): δ 8.53 (1H, br s, 4-OH), 8.27 (4H, br s, 11,13,11′,13′-OH), 7.42 (1H, d, *J* = 8.1 Hz, H-6′), 7.24 (1H, s, H-2′), 7.23 (2H, d, *J* = 8.5 Hz, H-2,6), 7.05 (1H, d, *J* = 16.3 Hz, H-7′), 6.89 (1H, d, *J* = 16.3 Hz, H-8′), 6.86 (1H, d, *J* = 8.1 Hz, H-5′), 6.84 (2H, d, *J* = 8.5 Hz, H-3,5), 6.52 (2H, s, H-10′,14′), 6.27 (1H, s, H-12), 6.24 (1H, s, H-12′), 6.18 (2H, s, H-10,14), 5.44 (1H, d, *J* = 8.0 Hz, H-7), 4.45 (1H, d, *J* = 8.0 Hz, H-8). Compound **16** was identified as resveratrol *trans*-dehydrodimer.

Compound **17**: Pale pink solid.IR ν_{max} cm^{-1}: 3281, 3021, 2927, 1594, 1485, 1143, 827. ESMS *m/z*: 453 $[M-H]^-$. ^{1}H NMR (400 MHz, CD_3COCD_3): δ 8.08 (2H, br s, 13,13′-OH), 8.06 (2H, br s, 4,14′-OH), 7.82 (2H, br s, 11,11′-OH), 6.97 (4H, d, *J* = 8.3 Hz, H-2,6,2′,6′), 6.69 (4H, d, *J* = 8.3 Hz, H-3,5,3′,5′), 6.61 (2H, s, H-14,14′), 6.18 (2H, s, H-12,12′), 4.56 (2H, s, H-7,7′), 3.80 (2H, s, H-8,8′). Compound **17** was identified as pallidol.

2.5 Biological Evaluations

All natural compounds and synthetic analogues were evaluated for cytotoxicity against HeLa, HT 29, and MCF-7 cell lines.

3. Results and Discussion

The structures of natural compounds and synthetic analogues were elucidated by spectroscopic methods and comparison with the spectroscopic data with those reported previously.

All compounds were tested for cytotoxicity against HeLa, HT 29, and MCF-7 cell lines. All natural compounds did not show cytotoxic activity at 50 μg/ml. For cytotoxic activity against HeLa cell lines, some methylated analogues and compound **16** exhibited low cytotoxicity with IC_{50} in range 26-30 μg/ml. For cytotoxic activity against HT 29 cell lines, some methylated analogues showed low cytotoxicity. For cytotoxic activity against MCF-7 cell lines, compounds **9**, **10** and **16** showed low cytotoxicity with IC_{50} in the range 29-44 μg/ml.

4. Conclusions

Investigation of the MeOH extract of the stems of *Dracaena loureiri* led to the isolation of four dihydrochalcones, **1-4**, and one stilbene, resveratrol (**5**). Resveratrol derivatives **6-15** and resveratrol dimers **16** and **17** were synthesized and identified. Some methylated analogues and resveratrol dimer (**16**) showed low cytotoxic activity against of HeLa, HT 29 and MCF-7 cell lines.

Table 1: ^{1}H NMR Data of Compounds **1-4**

Position	1[a]	2[a]	3[b]	4[b]
2	-	-	-	-
3	6.07 (*s*)	5.99 (*s*)	6.44 (*s*)	6.04 (*s*)
4	-	-	-	-
5	6.07 (*s*)	6.11 (*s*)	6.33 (*d*, 8.0)	6.00 (*s*)
6	-	-	6.97 (*d*, 8.0)	-
1′	-	-	-	-
2′	7.83 (*d*, 8.4)	7.83 (*d*, 8.5)	7.90 (*d*, 8.4)	7.93 (*d*, 8.6)
3′	6.77 (*d*, 8.4)	6.77 (*d*, 8.5)	6.90 (*d*, 8.4)	6.89 (*d*, 8.6)
4′	-	-	-	-
5′	6.77 (*d*, 8.4)	6.77 (*d*, 8.5)	6.90 (*d*, 8.4)	6.89 (*d*, 8.6)
6′	7.83 (*d*, 8.4)	7.83 (*d*, 8.5)	7.90 (*d*, 8.4)	7.93 (*d*, 8.6)
α	2.94 (*m*)	3.24 (*m*)	2.94 (*m*)	2.94 (*m*)
β	2.91 (*m*)	2.86 (*m*)	2.91 (*m*)	2.91 (*m*)
2-OMe	3.74 (*s*)	3.73 (*s*)	3.77 (*s*)	-
4-OMe	3.71 (*s*)	3.69 (*s*)	-	-
6-OMe	3.74 (s)	-	-	3.71 (s)

[a] Recorded in $CDCl_3$, [b] recorded in CD_3COCD_3

Table 2: ^{1}H NMR Data of Compounds **5-15**

Position	5	6	7	8	9	10	11	12	13	14	15
1	-	-	-	-	-	-	-	-	-	-	-
2	7.40 (*d*, 8.4)	7.49 (*d*, 8.5)	7.42 (*d*, 8.4)	7.51 (*d*, 8.5)	7.43 (*d*, 8.2)	7.52 (*d*, 8.5)	7.59 (*d*, 7.5)	7.83 (*d*, 8.4)	7.61 (*d*, 8.5)	7.33 (*d*, 8.5)	7.63 (*d*, 8.4)
3	6.82 (*d*, 8.4)	6.91 (*d*, 8.5)	6.83 (*d*, 8.4)	6.92 (*d*, 8.5)	6.84 (*d*, 8.2)	6.93 (*d*, 8.5)	6.10 (*d*, 7.5)	6.77 (*d*, 8.4)	7.12 (*d*, 8.5)	6.78 (*d*, 8.5)	7.13 (*d*, 8.4)
4	-	-	-	-	-	-	-	-	-	-	-
5	6.82 (*d*, 8.4)	6.91 (*d*, 8.5)	6.83 (*d*, 8.4)	6.92 (*d*, 8.5)	6.84 (*d*, 8.2)	6.93 (*d*, 8.5)	6.10 (*d*, 7.5)	6.77 (*d*, 8.4)	7.12 (*d*, 8.5)	6.78 (*d*, 8.5)	7.13 (*d*, 8.4)
6	7.40 (*d*, 8.4)	7.49 (*d*, 8.5)	7.42 (*d*, 8.4)	7.51 (*d*, 8.5)	7.43 (*d*, 8.2)	7.52 (*d*, 8.5)	7.59 (*d*, 7.5)	7.23 (*d*, 8.4)	7.61 (*d*, 8.5)	7.33 (*d*, 8.5)	7.63 (*d*, 8.4)
1′	-	-	-	-	-	-	-	-	-	-	-
2′	6.52 (*s*)	6.54 (*s*)	6.62 (*s*)	6.64 (*s*)	6.72 (*s*)	6.73 (*s*)	6.57 (*s*)	6.79 (*s*)	6.85 (*s*)	7.06 (*s*)	7.24 (*s*)
3′	-	-	-	-	-	-	-	-	-	-	-
4′	6.25 (*s*)	6.27 (*s*)	6.30 (*s*)	6.31 (*s*)	6.36 (*s*)	6.38 (*s*)	6.30 (*s*)	6.88 (*s*)	6.53 (*s*)	6.76 (*s*)	6.86 (*s*)
5′	-	-	-	-	-	-	-	-	-	-	-
6′	6.52 (*s*)	6.54 (*s*)	6.62 (*s*)	6.64 (*s*)	6.72 (*s*)	6.73 (*s*)	6.57 (*s*)	6.48 (*s*)	6.93 (*s*)	7.06(*s*)	7.24 (*s*)
α	7.00 (*d*, 16.4)	7.03 (*d*, 16.3)	7.08 (*d*, 16.3)	7.11 (*d*, 16.5)	7.17 (*d*, 16.3)	7.20 (*d*, 16.4)	7.09 (*d*, 16.0)	7.10 (*d*, 16.1)	7.20 (*d*, 16.0)	6.97 (*d*, 16.2)	7.30 (*d*, 16.3)
β	6.86 (*d*, 16.4)	6.92 (*d*, 16.3)	6.92 (*d*, 16.3)	6.97 (*d*, 16.5)	6.97 (*d*, 16.3)	7.02 (*d*, 16.4)	7.04 (*d*, 16.0)	6.94 (*d*, 16.1)	7.13 (*d*, 16.0)	6.82 (*d*, 16.2)	7.20 (*d*, 16.3)
4-OMe	-	3.79 (*s*)	-	3.77 (*s*)	-	3.80 (*s*)	-	-	-	-	-
3′-OMe	-	-	3.74 (*s*)	3.80 (*s*)	3.79 (*s*)	3.80 (*s*)	-	-	-	-	-
5′-OMe	-	-	-	-	3.79 (*s*)	3.80 (*s*)	-	-	-	-	-
4-OAc	-	-	-	-	-	-	2.24 (*s*)	-	2.24 (*s*)	-	2.25 (*s*)
3′-OAc	-	-	-	-	-	-	-	2.23 (*s*)	2.25 (*s*)	2.29 (*s*)	2.26 (*s*)
5′-OAc	-	-	-	-	-	-	-	-	-	2.29 (*s*)	2.26 (*s*)

Recorded in CD_3COCD_3

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