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Antimycobacterial flavonoids from *Derris indica*

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Abstract

Flavonoids (**1**–**4**), together with ten known compounds (**5**–**14**) were isolated from the stems and roots of the mangrove plant *Derris indica*. Their chemical structures were elucidated by analysis of their spectroscopic data. All compounds except compounds **2** and **6** exhibited antimycobacterial activity with minimum inhibitory concentrations (MIC) between 6.25 and 200 μg/mL.

Keywords: *Derris indica*; *Pongamia pinnata*; *Pongamia glabra*; *Cytisus pinnatus*; Leguminosae; Flavonoids; Antimycobacterial; *Mycobacterium tuberculosis*

1. Introduction

Derris indica (Lam.) Bennet (synonyms, *Pongamia pinnata* (L.) Pierre, *Pongamia pinnata* (L.) Merr., *Pongamia glabra* Vent. and *Cytisus pinnaus* (L.)) is a mangrove plant belonging to the Leguminosae family. *D. indica* is a medium sized, fast growing tree which is widely distributed in the region of Southeast Asia and Pacific Islands. The bark is thin gray to grayish-brown; leaves consist of 5 or 7 leaflets; flowers pinkish-white. Different plant parts of this plant have been used in folk medicine for bronchitis, whooping cough, rheumatic joints and to quench dipsia in diabetes (Yadav et al., 2004). During our search for antimycobacterial substances from Thai medicinal mangrove plants, we found that the crude hexane and dichloromethane extracts from the stems and roots of *D. indica* exhibited moderate antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra (Collins and Franzblau, 1997) with a minimum inhibition concentration (MIC) of 12.5–50 μg/mL. There are several earlier studies of biologically active substances, i.e., rotenoid (Simin et al., 2002), flavones (Tsuchiya et al., 1985; Simin et al., 2002), flavanones and chalcones (Carcache-Blanco et al., 2003, 2004) from *D. indica*; reviewed by Meera et al., 2003. However, no previous antimycobacterial activity in this plant has been reported. We report herein the bioassay-guided fractionation of the extracts from the stems and roots of *D. indica* which resulted in isolation of four new flavonoids (**1**–**4**) along with ten known compounds (**5**–**14**). All compounds were evaluated for their antimycobacterial activity against *M. tuberculosis* H37Ra (Collins and Franzblau, 1997).

2. Results and discussion

The hexane and dichloromethane extracts of the stems and roots of *D. indica* on repeated column chromatography over silica gel eluted with hexane and increasing polarity with CH_2Cl_2, EtOAc and MeOH yielded four new compounds (**1**–**4**) and ten known compounds (**5**–**14**).

Compound **1** was obtained as a pale yellow solid with a m.p. 104–106 °C, and gave a HREIMS molecular ion peak at *m*/*z* 452.1466 consistent with molecular formula $C_{25}H_{24}O_8$ (calc. 452.1471). The IR spectrum which exhibited absorption bands at 1746 (OC=O) and 1641 (C=O)

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cm^{-1}, and the UV spectrum (λ_{max} 238, 256 and 313 nm) was suggestive of a flavone skeleton (Markham, 1982). The ^{13}C NMR spectrum (Table 2) showed twenty-three signals for twenty-five carbons. Analysis of the ^{13}C DEPT spectrum of this compound suggested the presence of a carbonyl (δ 174.3, C-4), two acetyl groups (δ 169.9/20.6, AcO-3″ and 170.4/20.5, AcO-4″), two oxygenated methines (δ 70.7, C-3″ and 61.1, C-4″), one oxygenated quaternary carbon (δ 77.3, C-2″), two methyls (δ 25.6, Me_1-2″ and 21.7, Me_2-2″) together with fourteen sp^2 hybridised carbons. Comparison of the ^{13}C signals of **1** with those of karanjachromene (**13**, Table 2) (Shao et al., 2001) suggested that compound **1** has a flavonoid structure very similar to **13**. The ^1H NMR spectrum of **1** (Table 1) is typical of flavones with an unsubstituted phenyl B ring. The characteristic peaks appear at δ 7.98 (2H, *m*, H-2′, 6′) and δ 7.49 (3H, *m*, H-3′, 4′, 5′). In addition, signals for *ortho*-coupled protons at δ 8.18 (1H, *d*, $J = 9.0$ Hz, H-5) and 6.93 (1H, *d*, $J = 9.0$ Hz, H-6), two oxygenated methine doublets at δ 6.66 (1H, *d*, $J = 5.0$ Hz, H-4″) and 5.32 (1H, *d*, $J = 5.0$ Hz, H-3″), a methoxy group at δ 3.89 (3H, *s*, MeO-3), two acetyl groups (δ 1.88, 3H, *s*, AcO-4″ and 2.11, 3H, *s*, AcO-3″) and two tertiary methyl groups (δ 1.46, 3H, *s*, Me_1-2″ and 1.48, 3H, *s*, Me_2-2″) were present in the ^1H NMR spectrum of **1**. All ^{13}C and ^1H NMR spectroscopic data of **1** were assigned using the information provided by COSY, HMQC and HMBC spectroscopy. The observed HMBC correlation from H-3″ and H-4″ to the carbonyl carbons of the two acetyl groups and from both of the tertiary methyl groups to the oxygenated quaternary carbon (C-2″) (Fig. 1), is consistent with the presence of a 1,2-diacetyl-3-oxo-3-methylbutyl structural unit in the molecule of **1**. In an NOE experiment, irradiation of the methoxy protons at δ 3.89 enhanced the signals of H-2′ and 6′, thus placing the methoxy group at C-3. Comparison of the ^1H and ^{13}C NMR spectra of compound **1** and 5-methoxy-(3″,4″-dihydro-3″,4″-diacetoxy)-2″,2″-dimethylpyrano-(7,8:5″,6″)-flavone (**15**), another flavone previously isolated from the stem bark of *Pongamia pinnata* (Carcache-Blanco et al., 2003, 2004), revealed their close structural similarity differing only in the position of their methoxyl group. The relative stereochemistry of H-3″ and H-4″ was assigned by NOE experiments. The signal corresponding to H-3″ (δ 5.32) was enhanced upon irradiation at H-4″ (δ 6.66). It was therefore proposed that H-3″ and H-4″ were *cis*, identical to that found in **15**. Compound **1** was assigned as 3-methoxy-(3″,4″-dihydro-3″,4″-diacetoxy)-2″,2″-dimethylpyrano-(7,8:5″,6″)-flavone. Comparison of the specific rotation of **1** and **15** ($[\alpha]_D$ −24.0° and −26.0°, respectively) indicates they probably have the same absolute stereochemistry, which remains unassigned.

Table 1
^1H NMR spectroscopic data (δ, ppm) of compounds **1**–**3**, **13**[a] and **15**[b] in $CDCl_3$

Position	1	13	15	2	3	4
2	–	–	–	–	7.99 *s*	–
3	–	–	6.72 *s*	7.30 *s*	–	–
4	–	–	–	–	–	–
5	8.18 *d* (9.0)	8.03 *d* (8.5)	–	8.16 *d* (9.0)	8.01 *d* (8.7)	8.19 *d* (9.0)
6	6.93 *d* (9.0)	6.86 *d* (8.5)	6.35 *s*	7.55 *dd* (9.0, 0.5)	7.04 *d* (9.0)	6.97 *dd* (9.0, 2.4)
8	–	–	–	–	–	
2′	7.98 *m*	8.08 *m*	7.78 *m*	–	7.50 *d* (9.0)	6.93 *d* (2.4)
3′	7.49 *m*	7.63 *m*	7.47–7.49 *m*	6.67 *s*	6.97 *d* (8.7)	7.30 *s*
4′	7.49 *m*	7.63 *m*	7.47–7.49 *m*	–	–	–
5′	7.49 *m*	7.63 *m*	7.47–7.49 *m*	–	6.97 *d* (8.7)	–
6′	7.98 *m*	8.08 *m*	7.78 *m*	7.46 *s*	7.50 *d* (9.0)	6.68 *s*
2″	–	–	–	7.76 *d* (2.0)	4.72 *d* (6.6)	–
3″	5.32 *d* (5.0)	5.74 *d* (5.0)	5.30 *d* (4.6)	7.18 *dd* (2.0, 0.5)	5.52 *t* (6.6)	–
4″	6.66 *d* (5.0)	6.89 *d* (5.0)	6.63 *d* (4.6)	–	–	–
O–CH_2–O	–	–	–	6.06 *s*	–	–
O–CH_2–C	–	–	–	–	–	6.07 s
3-OMe	3.89 *s*	3.88 *s*	–	–	–	5.16 *s*
5-OMe	–	–	3.97 *s*	–	–	–
7-OMe	–	–	–	–	–	–
8-OMe	–	–	–	–	4.00 *s*	3.93 *s*
2′-OMe	–	–	–	3.93 *s*	–	–
4′-OMe	–	–	–	–	3.85 *s*	–
3″-OAc	2.11 *s*	–	2.13 *s*	–	–	–
4″-OAc	1.88 *s*	–	1.97 *s*	–	–	–
2″-Me_1	1.46 *s*	1.51 *s*	1.46 *s*	–	–	–
2″-Me_2	1.48 *s*	1.51 *s*	1.51 *s*	–	–	–
4″-Me_1	–	–	–	–	1.78 *s*	–
4″-Me_2	–	–	–	–	1.81 *s*	–

[a] Shao et al. (2001).
[b] Carcache-Blanco et al. (2003, 2004).

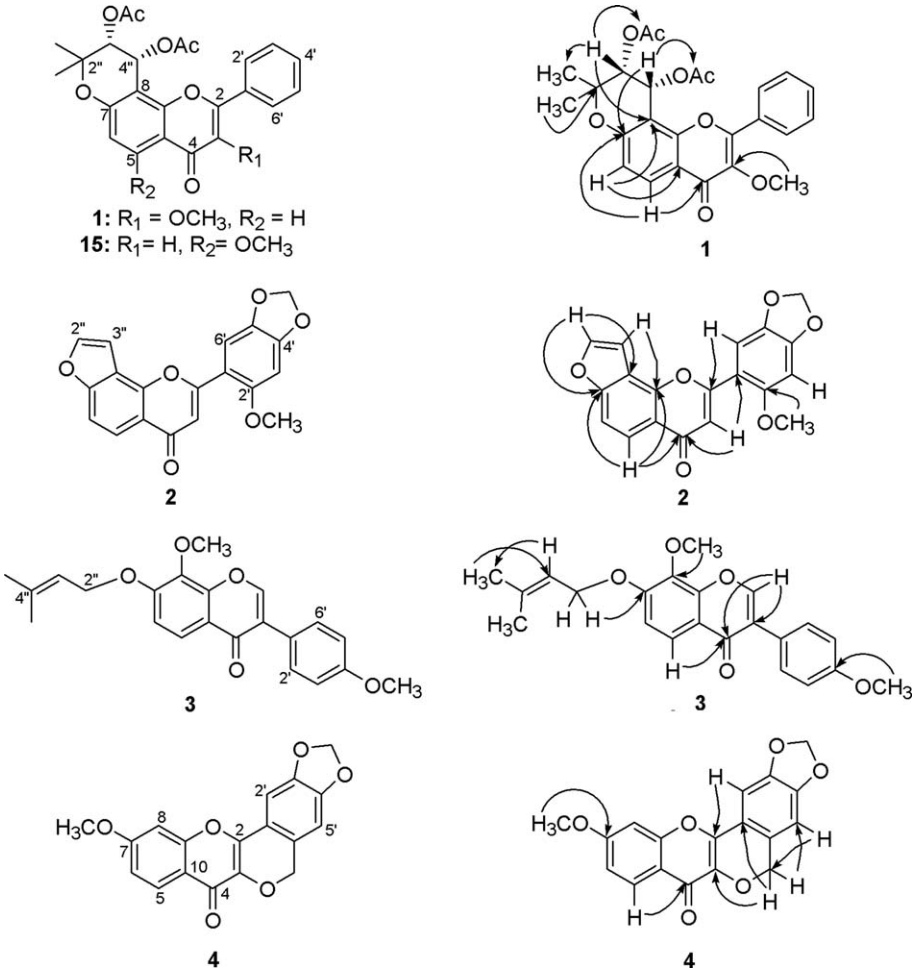

Fig. 1. Structures and selected HMBC correlations of compounds **1**–**4** and **15**.

Compound **2**, obtained as a yellowish gum, gave a molecular ion peak at *m*/*z* 336.0597 in its HREIMS corresponding to the molecular formula $C_{19}H_{12}O_6$ (calc. 336.0634). The IR band at 1609 (C=O) cm^{-1} and the UV absorption bands at 250, 297 and 350 nm were suggestive of a furanoflavone skeleton (Markham, 1982). This was supported by its ^1H NMR (Table 1) and ^{13}C NMR signals (Table 2) at δ 7.76 (1H, *d*, $J = 2.0$ Hz, H-2″)/δ 145.6 (C-2″) and δ 7.18 (1H, *dd*, $J = 2.0$, 0.5 Hz, H-3″)/δ 104.2 (C-3″) for a furan ring and the proton signals at δ 7.30 (1H, *s*) for H-3, δ 8.16 (1H, *d*, $J = 9.0$ Hz) and 7.55 (1H, *dd*, $J = 9.0$, 0.5 Hz) for H-5 and H-6, respectively. The HMBC correlations of H-2″ with C-7 and C-8 (Fig. 1) indicated that the furan ring should be fused at C-7 (oxygenated) and C-8 on ring A. The remaining ^1H NMR signals indicated that the ring B carried two protons, which showed their *para*-disposition by appearing as singlet signals at δ 6.67 and 7.46, a methoxyl group (δ_H 3.93 (1H, *s*); δ_C 56.5 (CH_3)) and a methylenedioxy moiety (δ_H 6.06 (2H, *s*); δ_C 102.1 (CH_2)). The presence of the two *para*-protons on ring B only allows the arrangement of substituents to be as shown. This substitution pattern was confirmed by NOE experiments. Irradiation of the signal at δ 6.67 (*s*, H-3′) enhanced the methoxyl signal at δ 3.93 (2′-OMe) which, when irradiated itself caused enhancement of the signal for the ring A hydrogen at δ 7.30 (*s*, H-3), as expected. In addition, the ^1H NMR spectrum obtained for compound **2** was closely comparable with that published for glabra II (Garg et al., 1978), the only difference being due to the change in position of the methoxy group. Thus, compound **2** was designated as 2′-methoxy-4′,5′-methylenedioxyfurano [7,8:4″,5″]-flavone.

Compound **3**, a yellow gum, exhibited a molecular ion peak at *m*/*z* 366.1467 in the HREIMS, indicating a molecular formula of $C_{22}H_{22}O_5$ (calc. 366.1467). The UV absorption band at 256 nm together with the IR spectrum (1643 (C=O) cm^{-1}) were indicative of an isoflavone skeleton (Markham, 1982). The ^{13}C NMR and DEPT spectra (Table 2) showed twenty signals for twenty-two carbons, corresponding to two methoxyl, two methyl, one methylene and eight methine groups and nine quaternary carbons. The ^1H NMR spectrum (Table 1) shows a characteristic of an isoflavone at δ 7.99 (1H, *s*) which is the proton at position 2. Two doublets ($J = 9.0$ Hz) at δ 7.50 and 6.97, each integrating for two protons, exhibit the characteristic AA′ XX′ pattern and was attributed to the 2′,6′- and 3′,5′-protons on ring B, respectively. The methoxy group (δ 3.85 (*s*)) was connected to C-4′ (δ

Table 2
^{13}C NMR spectroscopic data (δ, ppm) of compounds **1**–**3**, **13**[a] and **15**[b] in $CDCl_3$

Position	1	13	15	2	3	4
2	155.0	154.6	160.6	160.0	151.6	146.8
3	141.6	141.2	109.3	111.8	124.0	135.9
4	174.3	174.6	177.2	178.7	175.6	170.6
5	127.9	125.9	161.7	121.7	121.0	126.8
6	116.0	114.9	96.8	109.9	111.1	113.6
7	157.9	157.3	158.2	158.2	155.2	163.4
8	106.2	109.1	99.2	117.0	136.5	99.6
9	154.8	151.3	157.8	150.9	150.2	155.8
10	118.3	117.9	109.7	118.9	118.7	117.7
1′	130.5	130.2	131.8	112.7	123.7	118.8
2′	128.2	128.2	129.0	155.3	129.7	101.9
3′	128.5	131.1	125.9	95.0	113.5	147.8
4′	130.7	–	125.9[c]	151.1	159.1	149.7
5′	128.5	131.1	131.0[c]	141.8	113.5	105.5
6′	128.2	128.2	129.0	107.6	129.7	126.6
2″	77.3	77.7	77.3	145.6	65.8	–
3″	70.7	130.5	71.2	104.2	118.5	–
4″	61.1	115.0	61.3	–	138.4	–
O–CH_2–O	–	–	–	102.1	–	101.4
O–CH_2–C	–	–	–	–	–	67.8
3-OMe	60.2	60.1	–	–	–	–
5-OMe	–	–	56.5	–	–	–
7-OMe	–	–	–	–	–	55.3
8-OMe	–	–	–	–	61.0	–
2′-OMe	–	–	–	56.5	–	–
4′-OMe	–	–	–	–	54.8	–
3″-OAc	169.9, 20.6	–	169.8, 20.8	–	–	–
4″-OAc	170.4, 20.5	–	170.8, 20.7	–	–	–
2″-Me_1	25.6	20.8	26.3	–	–	–
2″-Me_2	21.7	20.8	21.5	–	–	–
4″-Me_1	–	–	–	–	17.8	–
4″-Me_2	–	–	–	–	25.3	–

[a] Shao et al. (2001).
[b] Carcache-Blanco et al. (2003, 2004).
[c] These assignments should be exchanged.

159.1) by their correlation in the HMBC experiment, and the correlation between H-2′/6′ (δ 7.50) and C-4′. The *ortho*-coupling of two protons at δ 8.01 (1H, *d*, $J = 9.0$ Hz) and 7.04 (1H, *d*, $J = 9.0$ Hz) in the ^{1}H NMR spectrum, and the HMBC correlation between the proton at δ 8.01 with the carbonyl carbon (C-4) suggested that they were H-5 and H-6, respectively. The ^{1}H NMR spectrum further showed the presence of a γ,γ-dimethylallyloxyl group (δ 1.78, 1.81 (3H, each *s*, CH_3), 4.72 (2H, *d*, $J = 6.6$ Hz, OCH_2) and 5.52 (1H, *br t*, $J = 6.6$ Hz, CH)). The position of the γ,γ-dimethylallyloxyl group was assigned by NOE experiments which displayed reciprocal enhancements between H-2″ and H-6. The second methoxyl group (δ 4.00, 3H, *s*) must therefore reside on carbon 8 on ring A. Furthermore, ^{1}H NMR spectroscopic data of compound **3** was closely comparable to that of maxima isoflavone J (Venkata Rao et al., 1994), with the only difference being due to the presence of the methoxy group at C-8 in compound **3**. Thus, the new compound **3** was assigned as 8,4′-dimethoxy-7-*O*-γ,γ-dimethylallylisoflavone.

Compound **4** was obtained as a yellow gum with a molecular formula of $C_{18}H_{12}O_6$, on the basis of the molecular ion at *m*/*z* 324.0606 in HREIMS (calc. 324.0634). The IR spectrum exhibits an absorbance at 1613 (C=O) cm^{-1} and the UV spectrum shows maxima at 240, 322, 346 and 359 nm which is suggestive of a flavone skeleton (Markham, 1982). The ^{13}C NMR and DEPT spectra of **4** displayed resonances for one carbonyl group (δ 170.6, C-4), fourteen olefinic carbons, one oxymethylene carbon (δ 67.8), one dioxymethylene (δ 101.4) and one methoxyl group at δ 55.3. The ^{1}H NMR spectrum of **4** revealed signals for an ABX spin system involving aromatic protons at δ 6.93 (1H, *d*, $J = 2.4$ Hz), 6.97 (1H, *dd*, $J = 9.0$, 2.4 Hz) and 8.19 (1H, *d*, $J = 9.0$ Hz) which were assigned to H-8, H-6 and H-5, respectively. The HMBC correlation between the proton signal at δ 8.19 and carbonyl carbon placed the carbonyl group *ortho* to H-5 (C-4). The ^{1}H and ^{13}C NMR spectra also exhibited one methoxyl signal which was placed at C-7 because of the upfield position of H-8 and the NOE observed between the methoxy protons and H-6 and 8. All carbon positions on ring A were confirmed by HMQC and HMBC experiments. This compound also exhibits characteristics of *para*-disposed aromatic protons at δ 7.30 (1H, *s*, H-2′) and 6.68 (1H, *s*, H-5′) in the ^{1}H NMR spectrum,

together with a methylenedioxy moiety at δ 6.07 (2H, *s*). The methylenedioxy group must be located at C-3′ and C-4′ in ring B. The ^{1}H and ^{13}C NMR spectra further show the signals of an oxymethylene group (δ_H 5.16 (2H, *s*)/δ_C 67.8 (CH_2)) which is attached at C-6′ (δ 126.6) by the HMBC correlations of oxymethylene protons to C-3, C-1′ and C-5′. Reciprocal enhancement of the proton at C-5′ and oxymethylene protons in NOE experiments support this orientation of the oxymethylene moiety. Thus, the oxygen of this moiety must be bonded to C-3 (δ 163.4). Compound **4** was assigned as 3,4-methylenedioxy-10-methoxy-7-oxo[2]benzopyrano[4,3-b]benzopyran.

The structures of the known isolated compounds (**5**–**14**) from *D. indica* (see Fig. 2) were determined from physical and spectroscopic data measurements and comparison with the relevant published data values as desmethoxy kanugin (**5**) (Das et al., 1994), karanjin (**6**) (Talapatra et al., 1980), lacheolatin B (**7**) (Tanaka et al., 1992), pongachromene (**8**) (Mukerjee et al., 1969), 3,7-dimethoxyflavone (**9**) (Tanaka et al., 1992), pachycarin D (**10**) (Shao et al., 2001), maackiain (**11**) (Garcez et al., 1988), medicarpin (**12**) (Afzail and Al-Oriquat, 1986), karanjachromene (**13**) (Garcez et al., 1988) and pinnatin (**14**) (Das et al., 1994).

The results of the antimycobacterial activity assays of the isolated compounds **1**–**14** are shown in Table 3. Compounds **1**, **4**, **5**, **7**, **9**, **11**, **13** and **14** exhibited moderate activity against *M. tuberculosis*, while compounds **3**, **8**, **10** and **12** exhibited weak antimycobacterial activity. No antimycobacterial activity was observed for compounds **2** and **6**.

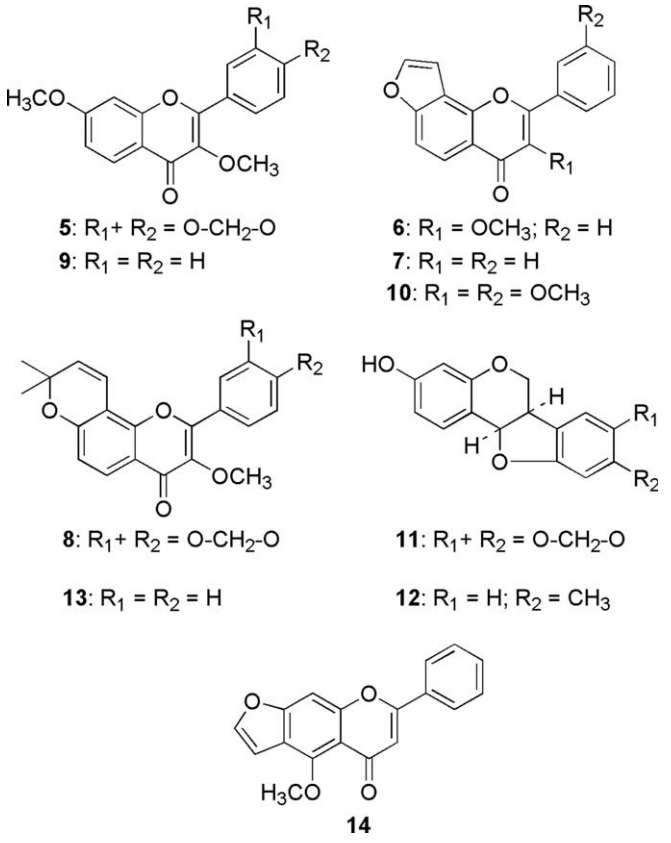

Fig. 2. Structures of compounds **5**–**14**.

2.1. Concluding remarks

Various extracts of *D. indica* have been reported to show biological activities such as antifeedant, antifungal, antibacterial, anti-inflammatory, antiulcer and antidiarrhoeal activities (Meera et al., 2003). The antimycobacterial activity against *M. tuberculosis* H37Ra is a new addition to the activities reported for this plant. In this paper we add four new members to the list of flavonoid compounds previously reported from this plant. This is only the second time that a pyranoflavone, in this case diacetoxypyranoflavone (**1**), has been isolated from *D. indica* (Carcache-Blanco et al., 2003). Although many methylenedioxyfuranoflavones have been isolated from this plant (Garg et al., 1978; Talapatra et al., 1982), compound **2** in which the methoxyl group is attached at C-2′ is a new derivative. It seems that oxygenated isoflavone derivatives are characteristic flavonoids of plants belonging to the *Derris* genus, with the presence of the 7-*O*-γ,γ-dimethylallylisoflavone derivative **3** in this plant being quite unusual. In addition, this is the first time that a peltogynan derivative (**4**) has been isolated from this plant. Three of the new compounds (compounds **1**, **3** and **4**) exhibit antimycobacterial activity.

Finally, it was thought that, with the concomitant isolation of 14 compounds which were all tested for antimycobacterial activity, it might be possible to make some inferences regarding structure–activity relationships. In the event, careful inspection of the molecular structures of the compounds and comparison of their activities did not reveal any useful or consistent trends.

Table 3
Antimycobacterial activity of compounds **1**–**14**

Compound	MIC (μg/mL)
1	25
2	Inactive
3	100
4	6.25
5	50
6	Inactive
7	50
8	100
9	50
10	200
11	50
12	100
13	12.5
14	12.5
Isoniazide[a]	0.040–0.090
Kanamycin sulfate[a]	2.0–5.0

[a] Positive controls.

3. Experimental

3.1. General

Specific rotations were determined with an ADP 220 polarimeter. UV and IR spectra were measured with UV Shimadzu 1601 and Perkin–Elmer 1750 FTIR spectrometers. Melting points were measured on an Electrothermal Melting Point Apparatus. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance DPX-300 MHz and Varian Unity INOVA 500 MHz spectrometers. Chemical shifts are recorded in parts per million (δ) downfield from TMS in $CDCl_3$. HREIMS were performed using a ThermoFinnigan MAT 95 XL mass spectrometer. Column chromatography was carried out using silica gel 60 GF_{254} (Merck) as the stationary phase. Silica gel 60 F_{254} precoated aluminum plates (0.2 mm, Merck) were used for TLC analysis.

3.2. Plant material

The stems and roots of *D. indica* were collected at the Mangrove Research Station in Nakhon Si Thammarat province, Thailand, in August, 2002 and identified by Dr. Chatchai Ngamriebsakul. A voucher specimen (number WU-0029) was deposited in the herbarium of the School of Science, Walailak University, Thasala, Nakhon Si Thammarat, Thailand.

3.3. Extraction and isolation

Air-dried powdered stems (22.0 kg) and roots (2.3 kg) of *D. indica* were extracted with hexane (2×40 l) and CH_2Cl_2 (2×40 l) at room temperature. Each extract was filtered and evaporated in vacuo. On the basis of TLC profiles, crude hexane and CH_2Cl_2 extracts of roots and stems were combined to give a brown gummy residue (87.51 g) which was subjected to speedy column chromatography over silica gel (Harwood and Laboratory, 1985) eluted with hexane and increasing polarity with CH_2Cl_2, EtOAc and MeOH to give 16 fractions. On the basis of the biological activity of these fractions, fraction 4 (10.41 g) was applied to a Sephadex LH-20 column (eluted with MeOH/CH_2Cl_2, 1:1) to give three fractions, which were further separated by column chromatography over Si gel (EtOAc/hexane (1:9) to MeOH/EtOAc (1:4)) to afford **3** (4.6 mg). Fraction 6 (4.58 g) was subjected to Sephadex LH-20 chromatography (eluted with MeOH/CH_2Cl_2, 1:1) and repeated column chromatography over Si gel (EtOAc/hexane (1:9) to MeOH/EtOAc (1:1)) yielded flavones **13** (9.0 mg), **6** (117.1 mg), **8** (10.3 mg), **9** (42.9 mg), **10** (15.2 mg) and **1** (30.8 mg), respectively. Fraction 7 (2.83 g) was subjected to Sephadex LH-20 (MeOH/CH_2Cl_2, 1:1) column chromatography, then further purified by column chromatography over Si gel (CH_2Cl_2/hexane (1:1) to 100% EtOAc) to give **7** (23.5 mg). Fraction 8 (2.48 g) was purified by washing with hexane to give **5** (1.21 g). Fraction 9 (7.39 g) was applied to Sephadex LH-20 (eluted with MeOH/CH_2Cl_2, 1:1) to yield **11** (14.5 mg) and **12** (24.1 mg). Fraction 12 (10.09 g) was separated by column chromatography on Sephadex LH-20 (MeOH/CH_2Cl_2, 1:1) to afford **14** (486.1 mg), **2** (6.3 mg) and **4** (7.2 mg).

3.4. 3-Methoxy-(3″,4″-dihydro-3″,4″-diacetoxy)-2″,2″-dimethylpyrano-(7,8:5″,6″)-flavone (*1*)

Pale yellow solid, m.p. 104–106 °C; $[\alpha]_D^{24}$ −24 ($CHCl_3$, *c* 0.71); UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 238 (3.75), 256 (3.74), 313 (3.97); IR ν_{max}^{neat} cm^{-1}: 1746, 1641, 1600; for ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$), see Tables 1 and 2; EIMS *m/z* (%): 452 $[M]^+$ (20), 450 (100), 332 (44), 294 (31); HREIMS *m/z*: 452.1466 ($[M]^+$, calc. for $C_{25}H_{24}O_8$, 452.1471).

3.5. 2′-Methoxy-4′,5′-methylenedioxyfurano [7,8:4″,5″]-flavone (*2*)

Yellowish gum; UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 250 (4.47), 297 (4.06), 350 (4.26); IR ν_{max}^{neat} cm^{-1}: 1609, 1569, 1505; for ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$), see Tables 1 and 2; EIMS *m/z* (%): 336 $[M]^+$ (95), 305 (40), 256 (38), 176 (82), 161 (100); HREIMS *m/z*: 336.0597 ($[M]^+$, calc. for $C_{19}H_{12}O_6$, 336.0634).

3.6. 8,4′-Dimethoxy-7-O-γ,γ-dimethylallylisoflavone (*3*)

Yellow gum; UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 255 (4.60); IR ν_{max}^{neat} cm^{-1}: 1643, 1609, 1513; for ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$), see Tables 1 and 2; EIMS *m/z* (%): 366 $[M]^+$ (15), 299 (100), 297 (88), 132 (35); HREIMS *m/z* 366.1467 ($[M]^+$, calc. for $C_{22}H_{22}O_5$, 366.1467).

3.7. 3,4-Methylenedioxy-10-methoxy-7-oxo[2]benzopyrano[4,3-b]benzopyran (*4*)

Yellow gum; UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 240 (4.36), 322 (4.22), 346 (4.22), 359 (4.23); IR ν_{max}^{neat} cm^{-1}: 1613, 1503; for ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$), see Tables 1 and 2; EIMS *m/z* (%): 324 $[M]^+$ (100), 323 (70), 178 (51), 151 (65), 149 (80); HREIMS *m/z* 324.0606 ($[M]^+$, calc. for $C_{18}H_{12}O_6$, 324.0634).

3.8. Biological evaluation

Bacteriostatic antimycobacterial activity was assessed against *M. tuberculosis* H37Ra using the Microplate Alamar Blue Assay (MABA) (Collins and Franzblau, 1997). The lowest compound concentration effecting an inhibition of $\geqslant$90% was considered the MIC. The drugs isoniazid and kanamycin sulfate were used as reference compounds in the antimycobacterial assay (Table 3).

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