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COUMARINS OF *MERRILLIA CALOXYLON*

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Key Word Index—*Merrillia caloxylon*; Rutaceae; isoprenylcoumarins; murrangatin; mexoticin; (−)-phebalosin; sibiricin; 7-methoxy-8-(1′,2′-dihydroxy-3′-methylbutyl)coumarin; merrillin.

Abstract—From the roots of *Merrillia caloxylon* the five isoprenylcoumarins (−)-sibiricin, (−)-phebalosin, (−)-murrangatin, (−)-mexoticin and merillin were isolated.

INTRODUCTION

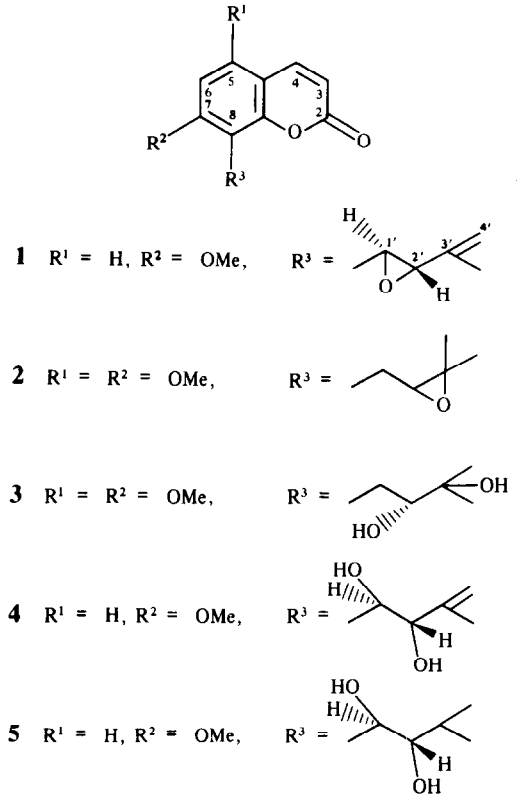
Merrillia caloxylon (Ridley) Swingle is a rare and endangered Rutaceae species of Peninsula Malaysia. Several flavonoids have been isolated from this plant [1–3]. Recently, the isoprenylcoumarins (−)-phebalosin (**1**) and (−)-sibiricin (**2**) along with indole and carbazole alkaloids have been found in this species [3]. These compounds are important as a chemotaxonomic markers connecting the genus *Merrillia* to the *Murraya* [3].

RESULTS AND DISCUSSION

Re-investigation on the root extracts of *M. caloxylon* revealed that the extracts are very rich in coumarins. TLC analysis showed the presence of at least 10 coumarin derivatives. Repeated column chromatography afforded five compounds among which the major one is (−)-phebalosin (**1**). This compound has recently been reported besides (−)-sibiricin (**2**) [3]. Two other known compounds isolated and identified are (−)-mexoticin (**3**) and (−)-murrangatin (**4**) which have also been previously isolated from several *Murraya* species, suggesting a close relationship between the *Murraya* and the *Merrillia* [4–6].

Acetylation of (−)-mexoticin (**3**) and (−)-murrangatin (**4**) gave the corresponding diacetates, which confirmed the presence of two hydroxy substituents for each compound. (−)-Phebalosin (**1**) was converted to (−)-murrangatin (**4**) by acid catalysed ring opening of the epoxide. In addition, the coupling constant of the two methine protons of (**4**) is 14 Hz establishes that they are in *anti* configuration.

From the $CHCl_3$ extract a new naturally occurring compound was isolated and given the trivial name merrillin (**5**). This compound gave an IR absorption band at 1710 cm^{-1} indicating the presence of a lactone carbonyl typical of a coumarin [7], and UV absorption bands characteristic of a 7-oxygenated-8-substituted coumarin (257 nm ($\log\varepsilon$ 3.75) and 323 (4.20) [8]). The mass spectrum gave the molecular ion $[M]^+$ 278 consistent with a molecular formula $C_{15}H_{18}O_5$. The ^1H NMR spectrum,



when compared with the ^1H NMR of other isoprenylcoumarins, (Table 1) suggests the structure 7-methoxy-8-(1′,2′-dihydroxy-3′-methylbutyl)coumarin. The characteristic doublet pairs for H-3/H-4 and H-5/H-6 suggest that the isoprenyl substituent has to be in the 8-position. Acetylation gave a diacetate which confirmed the presence of two hydroxy substituents on the isoprenyl side chain. (−)-Phebalosin (**1**), on treatment with dilute acid,

Table 1. ^{1}H NMR data for isoprenylcoumarins (200 MHz, $CDCl_3$)

H	1	2	3	4	5
3	6.25 *d* (9.5)*	6.13 *d* (9.5)	6.13 *d* (9.5)	6.25 *d* (9.5)	6.25 *d* (9.5)
4	7.64 *d* (9.5)	7.98 *d* (9.5)	7.98 *d* (9.5)	7.64 *d* (9.5)	7.64 *d* (9.5)
5	7.38 *d* (9.5)	3.92 (OMe)	3.92 (OMe)	7.38 *d* (9.5)	7.38 *d* (9.5)
6	6.86 *d* (9.5)	6.34 *s*	6.34 *s*	6.86 *d* (9.5)	6.86 *d* (9.5)
7-OMe	3.93	3.92	3.92	3.93	3.93
1′	5.28 *d* (2.1)		2.82 *dd* (14, 9.9)	5.29 *d* (8.4)	5.29 *d* (8.0)
		2.8–3.3 *m*	3.00 *dd* (2.7, 14)		
2′	5.06 *d* (2.1)		3.58 *dd* (2.7, 9.9)	4.5 *d* (8.4)	3.83 *dd* (3.5, 8.0)
3′	—	—	—		8.87 *m*
4′	3.93 *m*	—	—	4.59 *d* (13.2)	—
				4.60 *d* (13.2)	
Me	1.83 *s*	1.27 *s*	1.30 *s*	—	0.94 *d* (1.6)
					0.97 *d* (1.6)

*J in Hz.

afforded (−)-murrangatin (**4**) and subsequent hydrogenation gave 7-methoxy-8 (3′-methyl-1′,2′-dihydroxybutyl)coumarin whose spectral data are identical with that of merillin (**5**). The spectroscopic data obtained resemble to the literature data for dihydromurrangatin previously synthesized from murrangatin (**4**) [9].

EXPERIMENTAL

The plant material was provided by Mr Mustafa Mohamad (Rimba Ilmu, Botanic Garden, Universiti Malaya, Kuala Lumpur, Malaysia). Mps: uncorr; ^{1}H NMR: 200 MHz, $CDCl_3$, with TMS as an int. standard.

Isolation. Air-dried, powdered roots sample of *Merrillia caloxylon* (500 g) was consecutively extracted with hexane, $CHCl_3$ and finally with MeOH. The hexane extract on concn gave 710 mg of crude extract which on repeated CC (siilica gel) eluting with EtOAc afforded (−)-phebalosin (**1**) (320 mg), (−)-sibiricin (**2**) (10 mg), and (−)-mexoticin (**3**) (5 mg). The $CHCl_3$ extract on CC (silica gel) eluting with EtOAc gave merillin (**5**) (3 mg) in addition to (−) phebalosin (**1**), (−)-sibiricin (**2**), (−)-murrangatin (**4**) (8 mg) and (−)-mexoticin (**3**) (20 mg). The MeOH extract also gives the same compounds as above.

(−)-Phebalosin (**1**). Colourless needle-shaped crystals, mp 120° [lit. 120.5–121.5° (3)]; $[\alpha]^{20}$ −36° ($CHCl_3$; *c* 0.52). Spectral data identical with the published data [7].

(−)-Sibiricin (**2**). Colourless needle-shaped crystals, mp 152° [lit. 152° (3)]; $[\alpha]^{20}$ −67° ($CHCl_3$; *c* 0.50). Spectral data identical to the published data [8].

(−)-Mexoticin (**3**). Colourless needle crystals, mp 187° [lit. 185° (9)]; $[\alpha]^{20}$ −39° ($CHCl_3$; *c* 0.135). Spectral data identical to the published data [10, 11].

Mexoticin diacetate. (−)-Mexoticin (**3**) (10 mg) was dissolved in 2 ml Ac_2O–pyridine (1 : 2) and left to stand for 24 hr. Then, the excess Ac_2O was decomposed in H_2O and the products extracted with $CHCl_3$. The $CHCl_3$ portion was washed with water and concd to dryness. Repeated recrystallization gave 15 mg (−)-mexoticin diacetate mp 135°; IR (ν^{KBr}) cm^{-1}: 1723 (CO), 1713 (CO), 1604, 1438, 1373, 1330, 1250, 1135; ^{1}H NMR (200 Mz, $CDCl_3$) δ: 1.27 (3H, *s*, Me), 1.32 (3H, *s*, Me), 2.05 (6H, *s*, 2 OAc), 2.96 (1H, *dd*, J = 14.0, 2.6 Hz, H-1′), 3.13 (1H, *dd*, J = 14.0, 10.0 Hz, H-1′), 3.89 (3H, *s*, OMe at C-7), 3.91 (3H, *s*, OMe at C-6), 5.00 (1H, *dd*, J = 10.0, 2.6 Hz, H-2′), 6.13 (1H, *d*, J = 9.5 Hz, H-3), 6.26 (1H, *s*, H-6), and 7.98 (1H, *d*, J = 9.5 Hz, H-4). MS *m/z*: 392 $[M]^+$ (0.5), 350 (2), 332 (1), 290 (35), 275 (29), 259 (40), 219 (100), 206 (14).

(−)-Murrangatin (**4**). Viscous oil; $[\alpha]^{20}$ −15.0° ($CHCl_3$, *c* 0.770); UV λ^{EtOH} nm (log ε): 250sh. (3.65), 321 (4.20). IR ν^{KBr} cm^{-1}: 3350, 2960, 1730 (CO), 1605, 1563, 1498, 1287, 1120; MS *m/z*: 276 $[M]^+$ (2), 260 (2), 242 (10), 230 (4), 218 (21), 205 (100), 189 (12), 175 (8), 160 (6),149 (6), 143 (10), 130 (54).

Murrangatin diacetate. (−)-Murangatin (**4**) (3 mg) on acetylation as above gave murangatin diacetate, mp 50°; UV λ^{EtOH} nm (log ε): 248 (3.64), 258 (3.64), 321 (4.17); ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.60 (3H, *s*, Me), 2.01 (3H, *s*, OAc), 2.04 (3H, *s*, OAc), 3.91 (3H, *s*, OMe-7), 4.67 (1H, *t*, J = 1 Hz, H-4′); 4.88 (1H, *m*, H-4′), 6.04 (1H, *d*, J = 8.8 Hz, H-2′), 6.64 (1H, *d*, J = 8.8 Hz, H-1′), 6.25 (1H, *d*, J = 9.5 Hz, H-3), 6.86 (1H, *d*, J = 9.5, H-6), 7.38 (1H, *d*, J = 9.5, H-4), 7.64 (1H, *d*, J = 9.5 Hz, H-4); MS *m/z*: 360 $[M]^+$ (1), 309 (1), 300 (70), 258 (100), 247 (100), 241 (11).

Merrillin (**5**). A colourless plate, mp 68°; $[\alpha]^{20}$ −15.6° ($CHCl_3$; *c* 0.135); IR ν^{KBr} cm^{-1}: 3400, 2960, 1724 (CO), 1284, 1252, 1112, 1091, 1057, 1043, 1008, 835; MS *m/z*: 278 $[M]^+$ (2), 260 (1), 231 (1), 217 (4), 206 (100), 191 (45), 178 (10), 175 (8), 160 (7), 149 (1), 147 (2).

Merrillin diacetate. Merillin (**5**) (3 mg) was acetylated as above to afford a diacetate as an oil; ^{1}H NMR ($CDCl_3$) δ: 0.83 (1H, *m*, H-3′), 0.84 (3H, *d*, J = 2.2 Hz, Me), 0.87 (3H, *d*, J = 4.0 Hz, Me), 2.01 (3H, *s*, OAc), 2.03 (3H, *s*, OAc), 5.60 (1H, *dd*, J = 9.2, 1.5 Hz, H-2′), 6.84 (1H, *d*, J = 9.2 Hz, H-1′), 6.22 (1H, *d*, J = 9.8 Hz, H-3), 6.87 (1H, *d*, J = 9.5 Hz, H-6), 7.42 (1H, *d*, J = 9.5 Hz, H-5), 7.50 (1H, *d*, J = 9.8 Hz, H-4); MS *m/z*: 362 $[M]^+$ (8), 290 (19), 260 (4), 258 (42), 205 (100).

Synthesis of merrillin (**5**). (−)-Phebalosin (**1**) (100 mg) in 1 ml EtOH was mixed with 100 ml 0.5 M H_2SO_4 and stirred for 2 days at room temp. Then, the reaction mixture was extracted with $CHCl_3$ and the $CHCl_3$ extract was washed several times with water and finally concd to dryness. This gave (−)-murrangatin (**4**) (80 mg) after CC (silica gel) eluting with EtOAc. Hydrogenation of (−)-murrangatin (**4**) (10 mg) using 10% Pd/C at room temp. at 1 atm. for 1 hr gave merillin (**5**) after CC as above.

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ACETYLATED LIGNANS FROM *JUNIPERUS SABINA*

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Key Word Index—*Juniperus sabina*; Cupressaceae; lignans; acetyl-epipodophyllotoxin; acetyl-epipicropodophyllotoxin.

Abstract—Two new natural products, the acetates of epipodophyllotoxin and epipicropodophyllotoxin, were isolated from the lignan fraction of a *n*-hexane extract of the leaves of *Juniperus sabina*, along with deoxypodophyllotoxin, deoxipicropodophyllotoxin, (−)-deoxypodorhizon, β-peltatin A methyl ether and picropodophyllotoxin.

As a continuation of our research into the lignans occurring in species of Cupressaceae [1], we have started work on the isolation and identification of the lignans of *Juniperus sabina* L., from whose *n*-hexane extract we have isolated deoxypodophyllotoxin (**1**), deoxypicropodophyllotoxin (**2**), (−)-deoxypodorhizon (**3**), β-peltatin A methyl ether (**4**), acetylepipodophyllotoxin (**5**), acetyl-epipicropodophyllotoxin (**6**) and picropodophyllotoxin (**7**).

Compounds **1**–**4** and **7** were identified by direct comparison with authentic samples [1] or by comparison of spectroscopic data published by other authors [2–5]. Compounds **5** and **6** were identical to the acetylation products of epipodophyllotoxin (**9**) and epipicropodophyllotoxin (**8**) [2, 3, 6–9] and have not been hitherto described as natural products.

Saponification of **5** with Na_2CO_3/MeOH–H_2O over 7 hr occurred with epimerization at C-2 yielding **8**. Upon saponification under gentler conditions ($NaHCO_3$/MeOH–H_2O; 7 hr at room temperature), a mixture of epipodophyllotoxin (**9**) and unreacted **5** was obtained. However, when the reaction time was extended to 46 hr only **8** was obtained.

EXPERIMENTAL

General experimental procedures. Mps: uncorr; Optical rotations: $CHCl_3$; UV: EtOH; IR: CH_2Cl_2 soln; ^1H NMR (200.13 MHz) and ^{13}C NMR (50.3 MHz): $CDCl_3$ with TMS as int. standard.