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Flavanone, triterpene and chromene derivatives from the stems of *Paramignya griffithii*

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Abstract

Five compounds, including three that are novel, were isolated from the stems of *Paramignya griffithii* (Rutaceae). Two new and one known prenylated flavanones were characterized as 3′,4′-dihydroxy-7-methoxy-8-(3-methylbut-2-enyl)-furano(4″,5″:6,5)-flavanone, 3′,4′-dihydroxy-7-methoxy-8-(3-methylbut-2-enyl)-2″-(1-hydroxy-1-methylethyl)-furano (4″,5″:6,5)-flavanone and amoradicin. In addition, a triterpene, 3-oxo-tirucalla-7,24-diene-21-al, and a novel chromene, 6-(2-hydroxyethyl)-2,2-dimethyl-2*H*-1-benzopyran, were identified. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Paramignya griffithii; Rutaceae; Flavanones; Triterpene; Chromene

1. Introduction

Paramignya griffithii Hook. f. (Hooker, 1875), a climbing evergreen shrub, belongs to the family Rutaceae. In Thailand the stem has been used for the treatment of nose infections (Prakorb, herbalist, Songkla, Thailand, personal communication to C.W.). Previous investigations of the allied species *P. monophylla* have lead to the isolation of triterpenes from the fruit (Kumar et al., 1991) and pyranocoumarins from stem and root bark (Kumar et al., 1995, 1998). In this study the whole stem of *P. griffithii* has been investigated and three flavanones, one triterpene and one chromene isolated and characterized.

2. Results and discussion

A methanolic extract of the stem was fractionated by silica gel and Sephadex LH-20 column chromatography, resulting in the isolation of three flavanones. Compound **1** obtained as pale yellow needles was proved to be identical to amoradicin (5,3′,4′-trihydroxy-7-methoxy-6,8-di-(3-methylbut-2-enyl)-flavanone, which has previously been identified from *Amorpha fruticosa* (Leguminosae) (Rozsa et al., 1984).

The HR-EIMS of **2** showed a strong $[M]^+$ at m/z 394, suggesting the empirical formula $C_{23}H_{22}O_6$. The ^1H and ^{13}C NMR spectra (Table 1) revealed resonances typical of a flavanone with 3′,4′-substitution in the B-ring and a fully substituted A-ring, the substituents being a single methoxyl, a 3-methylbut-2-enyl side chain and an additional furan ring. In the EIMS fragmentation of **2** the ion corresponding to the B-ring was observed at m/z 136 ($C_8H_8O_2$), requiring dihydroxy substituents in that ring.

Placement of the substituents around the A-ring was aided by nOe experiments which revealed a strong interaction between the methoxyl and H-3′ of the furan system, so requiring placement of the methoxyl adjacent to the furan. This restricted the options to structure **2** and the 5-methoxy-furano-(4″,5″:6,7)-regioisomer. Distinction between these was achieved through an analysis of HMBC correlations (Table 1). These showed both the methoxyl protons and the H-1‴ protons of the 3-methylbut-2-enyl side chain had ^3J correlations with the same carbon, resonating at 157.4 ppm, and so must be adjacent to each other. This required the compound to be assigned structure **2**.

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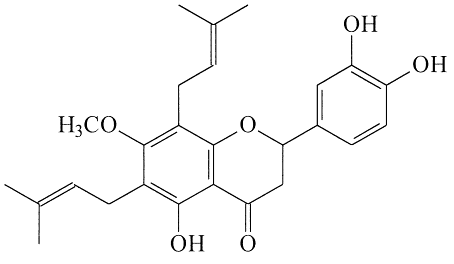

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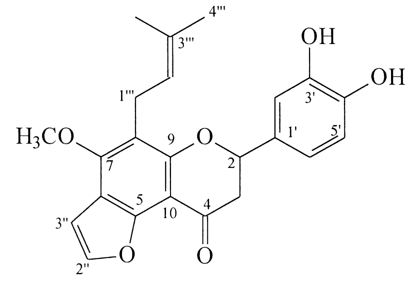

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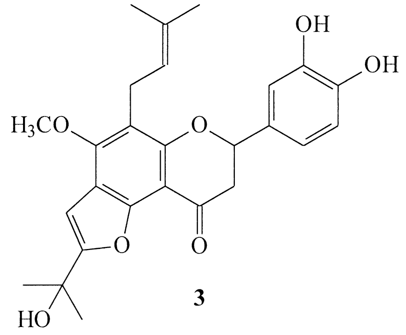

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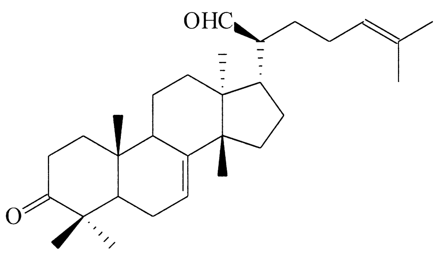

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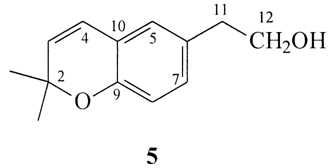

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The HR-EIMS of **3** showed an $[M]^+$ at m/z 452, suggesting the empirical formula $C_{26}H_{28}O_7$. The ^1H and ^{13}C NMR spectra (Table 2) were similar to **2**, the only difference being that **3** showed a 1-hydroxy-1-methylethyl (isopropanol) side chain substituted on the furan. In the EIMS fragmentation of **3** an ion was observed at m/z 434 ($C_{26}H_{26}O_6$), resulting from the loss of water from the 1-hydroxy-1-methylethyl side chain. Again nOe and HMBC experiments revealed the same pattern in the A-ring as in **2**. The methyl protons of the 1-hydroxy-1-methylethyl side chain had a ^3J correlation with the carbon resonating at 162.0 ppm, (C-2 of the furan), and so required this compound to be assigned structure **3**.

While the optical activity of **3** was found to be laevorotatory, typical for flavanones, that of **2** was found to be strongly dextrorotatory. We can offer no explanation for this unusual dichotomy as both samples appeared to be in excess of 95% pure by NMR.

The sequential partition of the methanolic extract with hexane and followed by chloroform gave compounds **4** and **5**. Compound **4** was isolated from the hexane extract and was obtained as colourless needles from a Me_2CO–$CHCl_3$ mixture and proved to be identical to 3-oxo-tirucalla-7,24-diene-21-al, which has previously been identified from *Simarouba amara* (Simaroubaceae) (Polonsky et al., 1976).

The HR-EIMS of **5** showed an $[M]^+$ at m/z 204, suggesting an empirical formula of $C_{13}H_{16}O_2$. The ^1H and $_{13}C$ NMR spectra (Table 3) revealed resonances typical of a 2,2-dimethylchromene (2,2-dimethylbenzopyran), with a 2-hydroxyethyl side chain substituted on the benzene ring. In the EIMS fragmentation of **5** a major ion at m/z 189 ($C_{12}H_{13}O_2$) could be attributed to the loss of a methyl group from the 2,2-dimethyl system. Placement of the 2-hydroxyethyl side chain was aided by nOe experiments. These showed that both the H-4 proton and the methylene protons of the 2-hydroxyethyl side

Table 1
NMR assignments and correlations for compound **2**[a]

				HMBC long-range correlations	
C/H	^{1}H	^{1}H[b]	^{13}C	^2J	^3J
2	5.41 *dd* (12.5, 3.2)	5.47 *dd* (12.6, 3.0)	79.6		
3	2.90 *dd* (16.8, 3.2) 3.06 *dd* (16.8, 12.5)	2.76 dd (16.7, 3.0) 3.06 *dd* (16.6, 12.6)	44.6	190.3	
4			190.3		
5			153.4		
6			112.2		
7			157.4		
8			115.2		
9			159.6		
10			104.1		
1′			131.9**[c]		
2′	7.03 *br s*	7.08 *d* (2.0)	113.7		79.6, 119.2, 144.1/2
′			144.1*		
4′			144.2*		
5′	6.91 *m*	6.87 *d* (8.1)	115.6		131.9/132.0
6′	6.91 *m*	6.92 *dd* (8.2, 2.0)	119.2	131.9/132.0	
2″	7.64 *d* (2.3)	7.78 *d* (2.3)	144.1		112.2, 153.4
3″	6.92 *d* (2.3)	7.16 *d* (2.3)	104.8	144.1	153.4
1‴	3.40 *d* (6.8)	3.38 *d* (7.1)	22.9	115.2, 122.7	131.9/132.0, 157.4, 159.6
2‴	5.17 *m*	5.18 *m*	122.7		
3‴			132.0**		
4‴	1.67 *s*	1.65 *s****	26.0	131.9/132.0	122.7
3‴-Me	1.67 *s*	1.62 *d* (1.0)***	18.0	131.9/132.0	122.7
OMe	4.20 *s*	4.22 *s*	60.0		157.4

[a] Spectra run in $CDCl_3$ at 400 MHz (^{1}H) and 100.56 MHz (^{13}C).

[b] Run in acetone-d_6.

[c] Resonances in the same column with the same number of (*) are interchangeable.

chain had interactions with the H-5 proton, so requiring placement of the 2-hydroxyethyl side chain adjacent to H-5. The HMBC correlations showed the 11-methylene protons of the 2-hydroxyethyl side chain had ^2J and ^3J correlations with carbons resonating at 64.0, 130.6, 127.0 and 129.7 ppm, requiring this compound to be assigned structure **5**.

Prenylated flavanones are the most abundant class of prenylated flavonoids with a rich variety of structures and occur mostly in the Leguminosae, Moraceae, Asteraceae and to a lesser extent in the Rutaceae, Euphorbiaceae and Scrophulariaceae (Barron and Ibrahim, 1996). In the Rutaceae this is the first time that flavonones have been reported from *Paramignya*. Indeed, the subtribe Triphasiinae, to which *Paramignya* belongs, is claimed to contain relatively few flavonoids (Grieve and Scora, 1980).

3. Experimental

3.1. General

Mp: uncorr., UV: MeOH, IR: KBr discs, ^{1}H and ^{13}C NMR [$CDCl_3$ (δ 7.27) or Acetone-d_6 (δ 2.05)] were run at 400 MHz and 100.56 MHz, respectively, residual solvent peaks as internal reference, EIMS: direct probe insert at 70 eV.

3.2. Plant material

Stems of *Paramignya griffithii* were collected from Songkla province, Thailand. A voucher specimen (number SKP. 1660301) has been deposited in the herbarium of the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand.

3.3. Extraction and isolation

Ground stems (8.26 kg) were extracted with MeOH, and yielded 484.7 g of crude extract. Part of this crude methanol extract (10.0 g) was subjected to column chromatography over silica gel, eluting with hexane followed by hexane containing an increasing amount of EtOAc. The fractions which eluted with 50–60% EtOAc were concentrated and subjected to column chromatography over Sephadex LH-20, eluting with $CHCl_3$ to give compound **1** (29.0 mg).

A further 25.0 g of the methanol extract was subjected to column chromatography over silica gel, eluting with hexane followed by hexane–EtOAc, EtOAc and

Table 2
NMR assignments for compound **3**[a]

C/H	^1H	^{13}C
2	5.28 *dd* (11.9,3.3)	79.5
3	2.80 *dd* (17.3,3.3) 2.96 *dd* (17.3,11.9)	44.1
4		191.4
5		153.0
6		112.5
7		157.9
8		115.1
9		159.5
10		103.6
1′		131.1[c]
2′	6.95 *br s*	113.6
3′		144.2
4′		144.8
5′	6.87 *d* (8.0)	115.5
6′	6.82 *br d* (8.2)	119.1
2″		162.0
3″	6.62 *s*	98.5
1‴	3.30 *d* (6.8)	22.8
2‴	5.10 *m*	122.6
3‴		131.9[c]
4‴	1.63 *s*	26.0
3‴-Me	1.63 *s*	18.0
OMe	4.14 *s*	59.9
C-O (hyd)[b]		68.9
Me_2 (hyd)[b]	1.69 *s*	28.3, 28.6

[a] Spectra run in $CDCl_3$ at 400 MHz (^1H) and 100.56 MHz (^{13}C).

[b] (hyd) = 1-Hydroxy-1-methylethyl side chain.

[c] Resonances in the same column are interchangeable.

Table 3
NMR assignments for compound **5**[a]

C/H	^1H	^{13}C
2		76.4
3	5.62 *d* (9.8)	131.2
4	6.30 *d* (9.8)	122.4
5	6.84 *d* (2.2)	127.0
6		130.6
7	6.96 *dd* (8.2, 2.2)	129.7
8	6.73 *d* (8.1)	116.6
9		151.8
10		121.5
11	2.78 *t* (6.5)	38.6
12	3.83 *td* (6.5, 5.9)	64.0
Me_2	1.43 *s*	28.2
OH	1.38 *t* (5.9)	

[a] Spectra run in $CDCl_3$ at 400 MHz (^1H) and 100.56 MHz (^{13}C).

EtOAc–MeOH. The fractions that eluted with 80% EtOAc in hexane to 2% MeOH in EtOAc were concentrated and subjected to column chromatography over Sephadex LH-20, eluting with $CHCl_3$ followed by 10% MeOH in $CHCl_3$. The fractions eluted with $CHCl_3$ were concentrated and repeatedly subjected to column chromatography over silica gel and Sephadex LH-20 to give compound **2** (2.7 mg). The fraction eluted with 10% MeOH in $CHCl_3$ gave compound **3** (10.6 mg).

A third sample (75.0 g) of crude methanol extract was dissolved in 50% MeOH. The sequential partition with hexane followed by $CHCl_3$, EtOAc and BuOH gave a hexane extract (7.2 g) and $CHCl_3$ extract (23.5 g). The hexane extract was subjected to column chromatography over silica gel. The fractions eluted with 12–14% EtOAc in hexane were concentrated and subjected to column chromatography over Sephadex LH-20, eluting with 33% CH_2Cl_2 in hexane. From this compound **4** (15.3 mg) was obtained. The $CHCl_3$ extract was partitioned (reflux) with hexane followed by EtOAc, Me_2CO and MeOH. The EtOAc extract (11.9 g) was concentrated and subjected to column chromatography over silica gel. The fractions eluted with 22–28% EtOAc in hexane were concentrated and repeatedly subjected to Sephadex LH-20 and silica gel column chromatography to give compound **5** (1.4 mg).

3.4. Amoradicin (*1*)

Yellow needles from MeOH, mp 60–65°C (Rozsa et al., 1984, 60–65°C). Found: M^+ 438.2030, $C_{26}H_{30}O_6$ requires 438.2034. The UV, IR, ^1H NMR, ^{13}C NMR and MS were in close agreement with previously published data (Rozsa et al., 1984).

3.5. 3′,4′-Dihydroxy-7-methoxy-8-(3-methylbut-2-enyl)-furano (4″,5″:6,5)-flavanone (*2*)

Amorphous yellow powder, $[\alpha]_D$ 122.22°C ($CHCl_3$, *c* 0.036), Found: M+ 394.1424; $C_{23}H_{22}O_6$ requires 394.1416. UV λ_{max} nm (log ε): 237 (4.3), 291 (4.1), 339 (sh). IR ν_{max} cm^{-1}: 3351, 1666, 1601. EIMS (rel.int.): 394 [M^+] (100), 351 (97), 339 (33), 257 (11), 215 (41), 203 (66), 136 (16). ^1H and ^{13}C NMR see Table 1.

3.6. 3′,4′-Dihydroxy-7-methoxy-8-(3-methylbut-2-enyl)-2‴-(1-hydroxy-1-methylethyl)-furano-(4″, 5″: 6, 5)-flavanone (*3*)

Amorphous yellow powder, $[\alpha]_D$–110.00°C (MeOH, *c* 0.040), Found: M^+ 452.1834; $C_{26}H_{28}O_7$ requires 452.1835. UV λ_{max} nm (log ϵ): 233 (sh), 289 (4.1), 337 (sh). IR ν_{max} cm^{-1}: 3407, 1657, 1600. EIMS (rel.int.): 452 [M+] (37), 434 (42), 383 (48), 247 (87), 192 (58). ^1H and ^{13}C NMR see Table 2.

3.7. 3-Oxo-tirucalla-7,24-diene-21-al (*4*)

Colourless needles from Me_2CO: $CHCl_3$, mp 155–156°C (Polonsky et al., 1976, 154–155°C). Found: M^+ 438.3400; $C_{30}H_{46}O_2$ requires 438.3498. The IR, ^1H

NMR, ^{13}C NMR and MS were in close agreement with previously published data (Polonsky et al., 1976; Mulholland et al., 1998).

*3.8. 6-(2-Hydroxyethyl)-2,2-dimethyl-2H-1-benzopyran (**5**)*

Pale yellow oil. Found M^+ 204.1160; $C_{13}H_{16}O_2$ requires 204.1150. UV λ_{max} (log ϵ): 261 (2.65), 312 (2.97). EIMS (rel. int.): 204 [M^+] (19), 190 (15), 189 (100), 173 (5), 171 (6), 159 (6), 158 (21), 107 (6). ^1H and ^{13}C NMR see Table 3.

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References

Barron, D., Ibrahim, R.K., 1996. Isoprenylated flavonoids — survey. Phytochemistry 43, 921–982.

Grieve, C.M., Scora, R.W., 1980. Flavonoid distribution in the Aurantioideae (Rutaceae). Systematic Botany 5, 39–53.

Hooker, J.D., 1875. The Flora of British India, Vol. I. L. Reeve and Co., Kent, (pp. 509–511)..

Kumar, V., Niyaz, N.M.M., Saminathan, S., Wickramaratne, D.B.M., 1998. Coumarins from *Paramignya monophylla* root bark. Phytochemistry 49, 215–218.

Kumar, V., Niyaz, N.M.M., Wickramaratne, D.B.M., 1995. Coumarins from stem bark of *Paramignya monophylla*. Phytochemistry 38, 805–806.

Kumar, V., Niyaz, N.M.M., Wickramaratne, D.B.M., Balasubramaniam, S., 1991. Tirucallane derivatives from *Paramignya monophylla* fruits. Phytochemistry 30, 1231–1233.

Mulholland, D.A., Kotsos, M., Mahomed, H.A., Taylor, D.A.H., 1998. Triterpenoids from *Owenia cepiodora*. Phytochemistry 49, 2457–2460.

Polonsky, J., Baskevitch-Varon, Z., Das, B.C., 1976. Triterpenes tetracycliques du *Simarouba amara*. Phytochemistry 15, 337–339.

Rozsa, Zs., Hohmann, J., Szendrei, K., Mester, I., Reisch, J., 1984. Amoradin, amoradicin and amoradinin, three prenylflavanones from *Amorpha fruticosa*. Phytochemistry 23, 1818–1819.