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GLEINENE AND GLEINADIENE, 5,7-DIMETHOXYCOUMARINS FROM *MURRAYA GLEINEI* ROOT

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Key Word Index—*Murraya gleinei*; Rutaceae; root; coumarins; sesquiterpenoids; fatty acids; gleinene; gleinadiene.

Abstract—*Murraya gleinei* root contained two new coumarins, 5,7-dimethoxy-8-(3′-methylbutan-1′,3′-dienyl)coumarin (gleinadiene) and 5,7-dimethoxy-8-(3′-methylbut-1′-enyl)coumarin (gleinene), together with eleven other coumarins, stigmasterol, bulnesol, guaiol, α-gurjunene and thirteen other sesquiterpenoids and six fatty acids.

INTRODUCTION

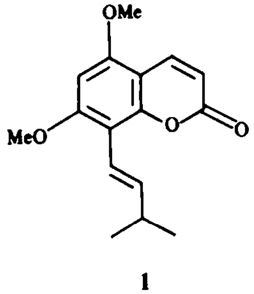
We have previously reported the isolation of several coumarins, the flavone exoticin, the alkaloid skimianine and the sterol stigmasterol, from the leaves of *Murraya gleinei*, a species endemic to Sri Lanka [1]. The root of *M. gleinei* contained several coumarins including the new coumarins gleinene (1) and gleinadiene (2). A diastereoisomer of murrangatin [2], which had previously been isolated from *M. paniculata* [3] and *Micromelum minutum* [4], together with 10 other coumarins—phebalosin, murrangatin, murralongin, sibiricin, mexoticin [1], murrayone [5], osthol [6], coumurrayin [7], omphamurin (3) [8], toddalenone [9], and the sterol stigmasterol—were also present. GC/MS analysis also showed the presence of 16 sesquiterpenoids and six fatty acids, the major constituent of the volatile fraction being bulnesol, guaiol and α-gurjunene.

RESULTS AND DISCUSSION

The dichloromethane extract of *M. gleinei* root bark on chromatographic separation gave stigmasterol and the coumarins phebalosin, sibiricin, murrangatin and mexoticin, which were present in the leaf [1]; murralongin, omphamurin, toddalenone, two new coumarins and a diastereoisomer of murrangatin. Mexoticin, phebalosin and sibiricin occurred as their laevorotatory forms, as was observed in the leaf [1]. Although the diastereoisomer of murrangatin isolated by us should be the *threo*-isomer, reported as murpanidin from *M. paniculata* [3] and minumicrolin from *Micromelum minutum* [4], and had similar spectral characteristics, there were inexplicable differences in the melting points and optical rotations of the samples isolated from the three sources.

The two new coumarins showed in their ^{1}H NMR spectra AB double doublets ($J = 10$ Hz) centred at $\sim\delta 6.15$ and $\sim\delta 8.0$ characteristic of the 3- and 4-protons of the coumarin ring. Two singlets, one at $\sim\delta 6.3$, due to one proton and the other at $\sim\delta 4.0$, due to six protons, suggested the presence of two methoxyl groups and one unsubstituted position in the aromatic ring. Analytical and spectroscopic data suggested that the coumarins had C_5H_9 and C_5H_7 side chains, respectively.

The ^{1}H NMR spectrum of the more polar coumarin showed signals for vinyl protons ($J = 16$ Hz) suggesting the presence of a *trans*-substituted double bond in its C_5H_7 side chain. A vinyl methyl signal at $\delta 2.0$ and a multiplet due to two vinylic methylene protons at $\delta 5.13$ indicated that the coumarin contained a 3′-methylbuta-1′,3′-dienyl side chain. Addition of Eu(fod)$_3$ shift reagent (1:1 molar ratio) caused the greatest shift (1.93 ppm) for the 3-H signal but those for the 1′-H signal (0.95 ppm) and the 2′-H signal (0.91 ppm) were significantly greater than those for the 4-H signal (0.65 ppm) and the aromatic



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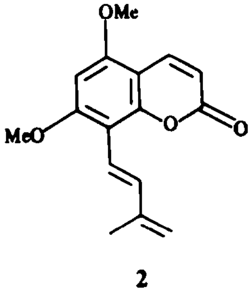

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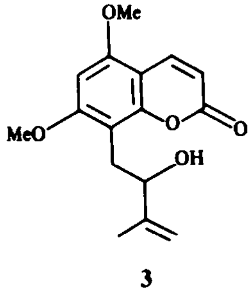

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singlet (0.46 ppm), confirming that the aromatic singlet was due to 6-H and that the side chain was at the 8-position. The dehydration of omphamurin (3) with $POCl_3$ gave this coumarin, confirming it to be 5,7-dimethoxy-8-(*E*-3′-methylbuta-1′,3′-dienyl)coumarin (gleinadiene) (2).

The ^{1}H NMR spectrum of the less polar coumarin contained a six-proton doublet at δ1.12 and a multiplet due to a single proton at δ2.50, indicating the presence of a Me_2CH– unit in its C_5H_9 side chain. A two-proton multiplet at δ6.63 ($W_{1/2}$ = 6 Hz) suggested the presence of a vinylic methylene group attached to the benzylic carbon atom. The vinylic protons in the alternative structure (1) would be expected to show an appreciable difference in chemical shift, as observed in 3-hydroxy-3-methylbut-1-enyl side chains [10], since one of them is benzylic. However, decoupling studies indicated that the CH proton of the isopropyl group was coupled with the vinylic protons. Irradiation at δ1.12 led to the collapse of the multiplet at δ2.50 into the X portion of an ABX system, while irradiation at δ2.50 not only transformed the doublet at δ1.12 into a singlet but also modified the multiplet at δ6.63. Experiments with Eu(fod)$_3$ shift reagent, which again indicated that the side chain was attached to C-8 with observed shifts decreasing in the order 3-H > 1′- and 2′-H > 4-H > 6-H > 3′-H, also made the vinyl proton signals amenable to analysis. These signals appeared as the AB part of an ABX system consisting of six lines made up of a lower field double doublet (J = 16.3 and 6.6 Hz), probably due to 2′-H and a higher field doublet (J = 16.3 Hz) whose lines were broadened suggesting it to represent 1′-H, showing long-range coupling with the 3′-H proton. Irradiation of the sample containing Eu(fod)$_3$ at the frequency of the 3′-H multiplet led to the collapse of the vinylic proton signals into an AB double doublet with J = 16.3 Hz, confirming that the side chain had a –CH=CH–CHMe$_2$ structure, the coupling constant indicating a *trans* stereochemistry for the double bond.

Comparison of the proton broad band and off-resonance decoupled ^{13}C NMR spectra of the coumarin revealed the presence of six quaternary carbon atoms, the low-field signals (δ150–162) being assigned to the carbonyl carbon and to 5-C, 7-C and 9-C while the signals at δ103.9 and 107.6 were assigned to 8-C and 10-C. Three methyl carbon signals were also present, the methoxyl carbons appearing at δ56.0 and 56.1, while the side-chain methyl carbons coincided at δ22.8. The remaining six signals appeared as doublets in the OFRD spectrum, the 3′-C at δ33.3, the 3-C, 4-C and 6-C at δ110.9, 138.9 and 90.6, respectively, and the 1′-C and 2′-C at δ143.0 and 115.0. The appearance of the side-chain vinylic carbons as doublets in the OFRD spectrum confirmed the presence of 3′-methylbut-1′-enyl side chain in the coumarin, which must have the structure 5,7-dimethoxy-8-(*E*-3′-methylbut-1′-enyl)coumarin (gleinene) (1).

The chloroform-soluble fraction of a methanol extract of *M. gleinei* root was partitioned between 90% methanol and hexane. The methanol layer was found to contain in addition to sibiricin, mexoticin, gleinadiene, murralongin, phebalosin and omphamurin, three more coumarins—murrayone, osthol and coumurrayin. GC/MS analysis of the hexane layer showed the presence of 30 compounds, which were identified as sesquiterpenoids, fatty acids and coumarins (Table 1). Bulnesol was the major sesquiterpenoid identified and together with guaiol and α-gurjunene, made up 65% of the volatiles in this layer.

EXPERIMENTAL

Mps are uncorr. Identities of compounds were established by mmp, IR, MS and NMR comparisons, unless otherwise stated. Petrol is the fraction 40–60°. Prep. TLC was carried out on Merck Kieselgel 60. Optical rotations were measured at 25° in $CHCl_3$. UV spectra were run in EtOH. IR spectra were recorded using KBr discs. ^{1}H NMR spectra were recorded at 60 MHz (Varian) or at 200 or 220 MHz (Bruker) with TMS as internal standard. Mass spectra were performed at MAT 44 at 70 eV. GC/MS was carried out on Varian MAT CH 7A, 70 eV using the GC parameters: FID, N_2 at 1.4 bar; temp. programmed from 80° to 270° at 9.7°/min; injector temp. 280°, detector temp. 290°; quartz capillary column OV-101, 50 mm i.d. (Macherey and Nagel), split valve 1:10.

M. gleinei was collected at Wilpattu in North West Sri Lanka; a voucher specimen has been deposited at the University herbarium.

Extraction. M. gleinei roots were debarked and the fresh bark (700 g) was extracted with CH_2Cl_2 for two 36 hr periods. Concn of the combined solns gave 63 g of CH_2Cl_2 extract. Fresh *M. gleinei* roots (1 kg) were also extracted with 90% MeOH; the extract was concentrated and shaken with $CHCl_3$. The $CHCl_3$ portion was divided into two parts, one of which was partitioned between 90% MeOH and *n*-hexane. The hexane layer was subjected to GC/MS analyses. The other portion of the $CHCl_3$ fraction was extracted with toluene to give the toluene extract.

Chromatography of the CH_2Cl_2 *extract.* The extract (30 g) was chromatographed on silica gel (550 g). Elution with petrol–EtOAc (49:1) gave a gum (8.2 g) which was shown by GC/MS to consist of a 1:1 mixture of bulnesol and guaiol. Repeated chromatography of the gum (1 g) on silica gel gave bulnesol (80 mg) and guaiol (65 mg), identical with authentic material, together with mixtures of varying proportions of the two compounds.

Elution with petrol–EtOAc (19:1) gave omphamurin (380 mg), needles from CH_2Cl_2–petrol, mp 130–131°, $[\alpha]_D - 22°$ (lit. [8], mp 131–132°, $[\alpha]_D - 22°$) and with petrol–EtOAc (9:1) gave stigmasterol, colourless, needles from MeOH–$CHCl_3$ (1.9 g), mp 166° $[\alpha]_D$ –49° (lit. [11], mp 168–170°, $[\alpha]_D$ –51°) and 5,7-dimethoxy-8-(3′-methylbut-1′-enyl)coumarin (gleinene) (1) (50 mg) which recrystallized from $CHCl_3$–petrol as colourless needless, mp 176–178° (found: C, 70.31; H, 6.28; $C_{16}H_{18}O_4$ requires: C, 70.05; H, 6.61%); IR ν_{max} cm^{-1}: 1700 and 1600; ^{1}H NMR (220 MHz): δ1.12 (*d*, J = 6.8 Hz, 6H, 2 × Me), 2.50 (*m*, $W_{1/2}$ = 21 Hz, 1H, 3′-H), 3.93 and 3.94 (*s*, 3H each, OMe), 6.15 (*d*, J = 9.7 Hz, 1H, 3-H), 6.32 (*s*, 1H, 6-H), 6.63 (*m*, $W_{1/2}$ = 6 Hz, 2H, 1′- and 2′-H), 7.98 (*d*, J = 9.7 Hz, 1H, 4-H); MS *m/z* (rel. int.): 274 $[M]^+$ (50), 259 (100) and 231 (40). ^{13}C NMR: δ22.8 (4′- and 5′-C), 33.3 (3′-C), 56.0, 56.1 (OMe-Cs), 90.6 (6-C), 103.9 (10-C), 107.6 (8-C), 110.9 (3-C), 115.0 (2′-C), 138.9 (4-C), 143.0 (1′-C), 155.3 (9-C), 161.1 and 161.5 (2-, 5- and 7-C).

Elution with petrol–EtOAc (17:3) gave 5,7-dimethoxy-8-(3′-methylbuta-1′,3′-dienyl)coumarin (gleinadiene) (620 mg), pale yellow cubes from petrol–EtOAc, mp 120–121° (found: C: 70.87; H, 5.99; $C_{16}H_{16}O_4$ requires: C, 70.57; H, 5.92%); HR-MS 272.1081 $[M]^+$; calc. for $C_{16}H_{16}O_4$: 272.1049; UV λ_{max} nm: 303, 277, 260, 233; IR ν_{max} cm^{-1}: 1720 and 1600; ^{1}H NMR: δ2.00 (*s*, 3H, Me), 3.96 (*s*, 6H, OMe), 5.13 (*m*, 2H, 4′-H), 6.13 (*d*, J = 10 Hz, 1H, 3-H), 6.30 (*s*, 1H, 6-H), 6.76 (*d*, J = 16 Hz, 1H, 2′-H), 7.46 (*d*, J = 16 Hz, 1H, 1′-H), 7.95 (*d*, J = 10 Hz, 1H, 4-H); MS *m/z* (rel. int.): 272 $[M]^+$ (100), 257 (10), 241 (51), 213 (35), 198 (12), 182 (10), 128 (11) and 95 (21).

Elution with petrol–EtOAc (4:1) gave a mixture (2.1 g) which consisted mainly of an unidentified long-chain fatty acid. Chromatography of this mixture on silica gel with $CHCl_3$–MeOH as eluant gave toddalenone (150 mg), pale yellow

Table 1. GC/MS analysis of the *n*-hexane extract of *Murraya gleinei* root

		MS		
Compounds	GC retention time (min.sec)	EI (100%) *m/z*	mass *m/z*	Constituent
1	17.20	81	204	β-Elemene
2	17.80	41	204	Caryophyllene
3	18.13	43	204	γ-Patchoulene
4	18.33	121	204	γ-Elemene
5	18.46	105	204	α-Guaiene
6	18.66	93	204	β-Selinene
7	19.40	161	204	γ-Cadinene
8	20.20	204	204	α-Gurjunene
9	20.40	69	204	β-Bisabolene
10	20.66	161	204	δ-Cadinene
11	22.46	161	222	Guaiol
12	22.80	161	204	β-Guaiene
13	24.13	135	222	Bulnesol
14	27.13	93	204	Humulene
15	28.80	105	204	Eremophilene
16	29.60	43	270	*n*-Heptadecanoic acid
17	29.86	149	278	Linolenic acid
18	31.33	73	256	Palmitic acid
19	33.20	43	220	(−)-β-Caryophyllene oxide
20	34.06	244	244	Osthol
21	35.93	67	280	Linoleic acid
22	36.20	55	282	Oleic acid
23	36.53	258	258	Phebalosin
24	36.86	41	284	Stearic acid
25	37.13	219	258	Murralongin
26	37.46	189	258	Murrayone
27	40.33	272	272	Gleinadiene
28	42.86	259	274	Coumurrayin
29	45.13	219	290	Sibiricin
30	45.80	219	290	Omphamurin

cubes from EtOAc, mp 239–240° (lit. [9], mp 244–246°), whose spectral characteristics were similar to those reported [9].

Elution with petrol–EtOAc (4:1) gave phebalosin (2.9 g), needles from CH_2Cl_2–petrol, mp 125–126°, $[\alpha]_D$ −43.6°; sibiricin (200 mg), mp 148–149°, $[\alpha]_D$ −59.7°; murrangatin (280 mg), mp 133°; and mexoticin (190 mg), mp 191–192°, $[\alpha]_D$ −31.1°, identical to those isolated from *M. gleinei* leaf [1]; together with murralongin, mp 134–135° (lit. [12], mp 135°), identical to an authentic sample, and *threo*-murrangatin, mp 142–143°, $[\alpha]_D$ +29.0 (lit. [3], mp 163–164°, $[\alpha]_D$ +14.6°, lit. [4] mp 132–135°, $[\alpha]_D$ +17.5°) (found: C, 65.19; H, 5.84; $C_{15}H_{16}O_5$ requires: C, 65.09; H, 5.79%); IR ν_{max} cm^{-1}: 3600, 1700 and 1600; ^1H NMR: δ1.88 (*br s*, 3H, Me), 2.2 (*br s*, 2H, OH), 4.00 (*s*, 3H, OMe), 4.53 (*d*, *J* = 8 Hz), 1-H, 2′-H), 5.00 (*m*, 2H, 4′-H), 5.43 (*br s*, *d*, *J* = 8 Hz on adding D_2O, 1H, 1′-H), 6.23 (*d*, *J* = 10 Hz, 1H, 3-H), 6.86 (*d*, *J* = 8 Hz, 1H, 6-H), 7.4 (*d*, *J* = 8 Hz, 1H, 5-H), 7.63 (*d*, *J* = 10 Hz, 1H, 4-H); MS *m/z* (rel. int.): 258 (77), 230 (42), 205 (100) and 175 (100).

Chromatography of the toluene extract. The toluene extract was applied on a silica gel column (Lichoprep Si 60, 440 × 37 mm, 63–125 μm, Merck) and eluted under pressure (1.6 bar) using toluene–EtOAc mixtures. In addition to sibiricin (162 mg), mexoticin (49 mg), gleinadiene (4 mg) and murralongin, phebalosin and omphamurin in small amounts, murrayone (125 mg), mp 129–132° (lit. [5], mp 130°); small amounts of osthol, mp 83–86° (lit. [6], mp 83–84°); and coumurrayin, mp 157–159° (lit. [7], mp 157–158°) were isolated and identified by mp and GC/MS comparisons.

GC/MS analysis of M. gleinei *root*. GC/MS analysis of the hexane extract showed the presence of the compounds given in Table 1. They were identified by GC, co-GC and literature comparisons [13, 14]. Bulnesol was the major sesquiterpenoid identified and bulnesol, guaiol and α-gurjunene accounted for 65% of the volatiles present in the hexane extract.

5,7-Dimethoxy-8-(3′-methylbuta-1′,3′-dienyl)coumarin (**2**). $POCl_3$ (0.5 ml) was added dropwise to a stirred soln of omphamurin (**3**) (14 mg) in pyridine (2 ml) at 25°. Work-up and purification by prep. TLC ($CHCl_3$) gave the coumarin (10 mg), identical with gleinadiene isolated above.

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