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PRENYLATED FLAVANONES FROM LEAVES OF *MACARANGA PLEIOSTEMONA*

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Key Word Index *Macaranga pleiostemona*; Euphorbiaceae; leaves; prenylflavanones; macarangaflavanones A and B; euchrestaflavanone A; bonannione A; antibacterial activity.

Abstract—From the dichloromethane extract of leaves of *Macaranga pleiostemona*, four antibacterial prenylated flavanones were isolated. Their structures were elucidated by analysis of their spectral data. One, macarangaflavanone A, is a new compound, the remaining three are previously reported natural products. Macarangaflavanone B is clearly shown not to be euchrestaflavanone A. Euchrestaflavanone A and lespedezaflavanone B are revealed to be identical natural products.

INTRODUCTION

Macaranga pleiostemona is an erect shrub or tree endemic to New Guinea in regrowth areas and at forest margins [1]. Contact with the leaves of this species produces, in some individuals, an allergic dermatitis-like reaction [1]. Local healers apply fresh or dried leaves of many *Macaranga* species to fresh cuts, sores, swellings, bruises and boils [2–4]. This plant species is also used to relieve headache, leaves being heated and applied directly to the forehead [3, 4]. Chemical investigations have been made on various *Macaranga* species; previously, a prenylated compound, the geranyl-substituted flavonol, macarangin, has been isolated from *M. vedeliana* [5].

In the present study, the petrol, dichloromethane (DCM), ethyl acetate, methanol and methanol–water (4:1) extracts of air-dried leaves of *M. pleiostemona* were screened for their potential biological activities. The DCM extract was found to exhibit significant antibacterial activity. The current report describes the isolation and structural elucidation of the new antibacterial active flavanone, macarangaflavanone A (**1**), as well as of the three known compounds macarangaflavanone B (**2**), euchrestaflavanone A (**3**) and bonannione A (**4**) [6–10].

RESULTS AND DISCUSSION

Bioactivity-guided fractionation of the DCM extract of air-dried leaves by silica gel vacuum liquid chromatography (VLC) and medium-pressure liquid chromatography (MPLC), followed by reverse-phase HPLC, as described in the Experimental, led to the isolation of the four substituted flavanones (**1**–**4**).

Compound **1** was obtained as a greenish oil, which analysed by HR EI mass spectrometry for $C_{25}H_{28}O_5$. Its UV (λ_{max}^{MeOH} nm = 286, 320 (sh)), ^{1}H NMR [δ2.37 (1H, *dd*, J = 17.1, 3.0 Hz, H_{eq}-3), 2.63 (1H, *dd*, J = 17.1, 13.0 Hz, H_{ax}-3), 4.76 (1H, *dd*, J = 13.0, 3.0 Hz, H-2), 12.83 (*s*, OH-5)] and ^{13}C NMR data (see Table 2 below) indicated it to be a flavanone derivative. Positive UV-shifts after the addition of sodium methanolate, sodium acetate and aluminium chloride showed the hydroxyl groups at C-5 and C-7 to be free [11]. The substitution pattern of ring B was analysed by ^{1}H NMR [δ6.54 (1H, *d*, J = 8.2 Hz, H-5′), 6.90 (1H, *dd*, J = 8.2, 2.3 Hz, H-6′), 7.05 (1H, *d*, J = 2.3 Hz, H-2′)] and 2D ROESY, which revealed NOE interactions between H-6′ and H-2 and H_2-3, and between and H-2′ and H-2 and H_2-3, as being 1, 3 and 4. The ^{1}H NMR spectrum also indicated the presence of a geranyl moiety [δ1.56, 1.64, 1.71 (each 3H, *s*, CH_3)], 3.33 (2H, *d*, J = 7.2 Hz, ar–CH_2–CH=), 5.18 and 5.38 (each 1H, *br t*, J = 6.8 and 7.2 Hz, CH_2–CH=), 2.06 and 2.15 (each 2H, *m*, H-5″ and H-6″). Since the 2D ROESY spectrum showed NOE interactions between H_2-1″ and H-2′, the geranyl group was assigned to C-3′. This deduction was further supported by the EI mass spectrum of **1**, in which two prominent fragment ions were observed at *m/z* 255 and 153, caused by a retro-Diels–Alder (RDA) fragmentation [12], indicating the geranyl moiety and the third hydroxyl group to be on ring B. The one chiral centre within **1** was assigned the *S*-configuration on the basis of its negative optical rotation and literature pre-

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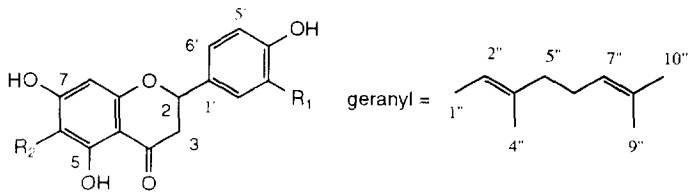
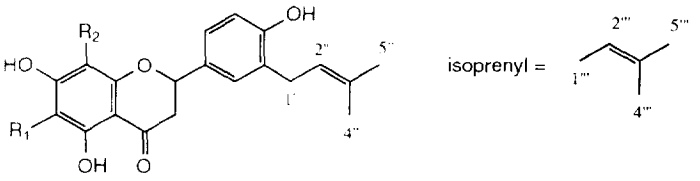


Compound	R_1	R_2
1	geranyl	H
4	H	geranyl



Compound	R_1	R_2
2	isoprenyl	H
3	H	isoprenyl

cedents [13]. From the above analysis, the structure of **1**, for which the trivial name macarangaflavanone A is proposed, was concluded to be 3'-geranyl-4',5,7-trihydroxyflavanone.

Compound **2** was also obtained as a greenish oil and found by HR EI mass spectrometry to have the molecular formula $C_{25}H_{28}O_5$. From its UV [λ_{max}^{MeOH} nm = 293, 332 (sh)], ^{1}H NMR [δ 2.77 (1H, *dd*, J = 17.2, 3.1 Hz, Hβ-3), 3.08 (1H, *dd*, J = 17.2, 13.1 Hz, Hα-3), 5.30 (1H, *dd*, J = 13.1, 3.1 Hz, H-2), 12.40 (*s*, OH-5)] and ^{13}C NMR spectra it was evident that it too was a substituted flavanone. The positive UV-shifts observed after the addition of sodium methanolate and sodium acetate indicated the hydroxyl groups at C-5 and C-7 to be free [11]. The ^{1}H NMR spectrum further displayed resonances indicative of two 3-methyl-2-butenyl (*iso*prenyl) groups [δ 1.78 (12H, *s*, $CH_3 \times 4$), 3.35 (2H, *d*, J = 7.6 Hz, ar-C$\underline{H}_2$-CH=) and 3.38 (2H, *d*, J = 7.1 Hz, ar-C$\underline{H}_2$-CH=), 5.27 and 5.32 (each *m*, CH_2-C$\underline{H}$=)]. The ^{13}C NMR resonances at δ 106.8 (*s*) and 95.5 (*d*) indicated that one of the A-ring carbons was substituted when compared to **1** (see Table 2), as did the chemical shift for O$\underline{H}$-5 (δ 12.40) [14]. The absence of a bathochromic shift in the UV spectrum of **2**, after the addition of aluminium chloride, indicated the substituent to be at C-6 [15]; one 3-methyl-2-butenyl group is located at C-6. Since the ^{1}H NMR spectrum of **2** displayed the characteristic signal pattern for an 1',3',4'-substituted aromatic ring (B-ring), the other 3-methyl-2-butenyl group must thus be at C-3', as in **1**. Further support for these deductions was found in the EI mass spectrum of **2**, which contained two prominent fragment ions at *m/z* 220 and 188, consistent with a RDA fragmentation [11], indicating one *iso*prenyl moiety to be on ring A, and the other and one hydroxyl group to be on ring B. From this analysis, the structure of **2** was concluded to be 4',5,7-trihydroxy-3',6-*bis*(3-methyl-2-butenyl) flavanone.

The ^{1}H NMR spectral data of **2** are essentially the same as those of euchrestaflavanone A [9], the structure of which has, since its first publication [6], been revised and shown to be 4',5,7-trihydroxy-3',8-*bis*(3-methyl-2-butenyl) flavanone [7]. Hence, 4',5,7-trihydroxy-3',6-*bis*(3-methyl-2-butenyl) flavanone, isolated from *Schoenus nigricans* [9], and *Lotus creticus* [16] is not euchrestaflavanone A. For this reason, it is proposed that 4',5,7-trihydroxy-3',6-*bis*(3-methyl-2-butenyl) flavanone be renamed as macarangaflavanone B (**2**).

Compound **3** was obtained as a green oil whose UV, IR, mass and ^{1}H NMR [7] spectral data were consistent with those of 4',5,7-trihydroxy-3',8-*bis*(3-methyl-2-butenyl) flavanone, isolated from *Euchresta japonica* and reported as euchrestaflavanone A [7]. This compound was subsequently isolated from *Lespedeza davidii* and called lespedezaflavanone B [8]. Hence, euchrestaflavanone A and lespedezaflavanone B are the same compound. For **3**, complete and unambiguous ^{1}H and ^{13}C NMR data are now reported in Tables 1 and 2 [17, 18], as they were found to be either incompletely reported [8] or recorded in different solvents [7, 19] previously. The ^{1}H NMR spectrum of compound **3** was unambiguously compared to the spectrum of an authentic sample of the same compound.

Compound **4** was identified as bonnanione A. Its spectral data (^{1}H NMR, ^{13}C NMR and EI mass spectra) were

Table 1. ^{1}H NMR data of flavanones **1**–**3** [$CDCl_3$, 300 MHz, coupling constants J(Hz)]

Proton	1*	Proton	2	3
2	4.76 *dd* (3.0, 13.0)	2	5.30 *dd* (3.1, 13.1)	5.33 *dd* (3.1, 12.9)
3_{ax}	2.63 *dd* (13.0, 17.1)	3_{ax}	3.08 *dd* (13.1, 17.2)	3.05 *dd* (12.9, 17.1)
3_{eq}	2.37 *dd* (3.0, 17.1)	3_{eq}	2.77 *dd* (3.1, 17.2)	2.77 *dd* (3.1, 17.1)
OH-5	12.83 *s*	5	12.40 *s*	12.01 *s*
6	6.00 *d* (1.9)†	6		6.02 *s*
8	5.96 *d* (1.9)†	8	6.00 *s*	
2′	7.05 *d* (2.3)	2′	7.18 *d* (1.9)	7.17 *d* (2.2)
5′	6.54 *d* (8.2)	5′	6.84 *d* (8.2)	6.85 *d* (8.1)
6′	6.90 *dd* (2.3, 8.2)	6′	7.19 *dd* (1.9, 8.2)	7.19 *dd* (2.2, 8.1)
1″	3.33 *d* (7.2)	1″	3.38 *d* (7.1)§	3.39 *d* (7.3)**
2″	5.38 *br t* (7.2)‡	2″	5.32 *m*¶	5.33 *m***
4″	1.64 *s*	4″	1.78 *s*	1.79 *s*
5″	2.06 *m*§	5″	1.78 *s*	1.79 *s*
6″	2.15 *m*§	1‴	3.35 *d* (7.6)§	3.31 *d* (7.3)**
7″	5.18 *br t* (6.8)‡	2‴	5.27 *m*¶	5.21 *m***
9″	1.56 *s*	4‴	1.78 *s*	1.79 *s*
10″	1.71 *s*	5‴	1.78 *s*	1.79 *s*

* In benzene-d_6.

†, ‡, §, ¶ Values interchangeable.

** Assigned from 2D ROESY spectrum which showed NOE interactions between H-1″ and H-2′, H-1″ and H-2″, H-1″ and H-4″ rsp. H-5″, H-2″ and H-2′, H-2″ and H-4″ rsp. H-5″; and between H-1‴ and H-2‴, H-1‴ and H-4‴ rsp. H-5‴, H-2‴ and H-4‴ rsp. H-5‴.

Table 2. ^{13}C NMR data of flavanones **1**–**3** ($CDCl_3$, 75 MHz)

Carbon	1*	Carbon	2	3
2	79.2 *d*†	2	79.0 *d*	78.9 *d*
3	43.2 *t*	3	43.3 *t*	43.2 *t*
4	196.3 *s*	4	196.3 *s*	196.5 *s*
5	165.1 *s*‡	5	161.2 *s*‡	162.3 *s*‡
6	96.8 *d*§	6	106.8 *s*	96.8 *d*
7	165.2 *s*‡	7	163.7 *s*‡	163.6 *s*‡
8	95.4 *d*§	8	95.5 *d*	106.0 *s*
9	163.8 *s*‡	9	161.2 *s*‡	159.8 *s*‡
10	103.6 *s*	10	103.0 *s*	103.0 *s*
1′	131.0 *s*	1′	130.6 *s*¶	130.8 *s*
2′	128.6 *d*	2′	128.2 *d*	128.0 *d*
3′	131.0 *s*	3′	127.3 *s*¶	130.8 *s*
4′	155.1 *s*	4′	154.9 *s*	154.7 *s*
5′	115.9 *s*	5′	116.0 *d*	115.9 *d*
6′	125.9 *d*	6′	125.7 *d*	125.5 *d*
1″	29.7 *t*	1″	29.9 *t*	29.8 *d*
2″	122.2 *d*	2″	121.4 *d*‖	121.6 *d*
3″	138.0 *s*	3″	135.7 *s***	135.4 *s*††
4″	16.1 *q*	4″	17.9 *q*‡‡	17.8 *d*‡‡
5″	40.0 *t*	5″	25.8 *d*‡‡	25.8 *q*‡‡
6″	26.8 *t*	1‴	21.1 *t*	21.8 *d*
7″	124.4 *d*	2‴	121.2 *d*	121.2 *d*
8″	131.7 *s*	3‴	135.4 *s***	135.1 *s*††
9″	17.7 *q*‡‡	4‴	17.9 *q*‡‡	17.8 *q*‡‡
10″	25.8 *q*‡‡	5‴	25.8 *q*‡‡	25.8 *q*‡‡

†Multiplicities by DEPT.

*In benzene-d_6.

‡Data assigned by comparison with ref. [17].

§, ¶, ‖Values interchangeable.

‡‡Data assigned by comparison with ref. [18].

in good agreement with those published [10]. For this compound, as was the case for compounds **1**–**3**, all of the reported ^{1}H and ^{13}C NMR assignments were confirmed from the results of extensive 2D NMR correlation measurements, including ^{1}H–^{1}H NMR COSY, ^{1}H ^{13}C NMR HMQC and HMBC.

The antibacterial potency of all compounds (**1**–**4**) was assessed against *Escherichia coli* and *Micrococcus luteus* [20]. All were shown to have significant antibacterial activities consistent with the traditional uses of this plant species [2–4] (Table 3).

EXPERIMENTAL

General. Silica gel 40–63 μm (Merck) was used for silica gel VLC and 15 μm for MPLC. HPLC was performed with a 16 mm × 250 mm column packed with reverse-phase material (Spherisorb S5 ODS II (Knauer)). NMR spectra were recorded at a basic frequency of 300 MHz: solvent resonances were used as int. refs. EIMS were measured at 70 eV.

Plant material. Leaves of *M. pleiostemona* Pax & K. Hoffm. were collected in April 1991, near Mendi, Papua New Guinea. A voucher specimen has been deposited at the Rijksherbarium (ETH 91/13 1-04-91), University of Leiden (The Netherlands).

Bioassays. Crude extracts, chromatographic frs and pure compounds were assayed for antibacterial activity against *Escherichia coli* (ATCC 25922) and *Micrococcus luteus* (ATCC 9341), using a bioautographic method [20].

Table 3. Antibacterial activities of compounds **1–4**

Compound	Minimum growth inhibition amount in μg on TLC	
	E. coli	*M. luteus*
1	0.5	0.5
2	0.5	0.5
3	0.5	0.5
4	0.5	0.5
Chloramphenicol	0.05	0.05

Extraction and isolation. Air-dried leaves (1500 g) were continuously percolated with *n*-hexane followed by CH_2Cl_2, EtOAc, MeOH and then MeOH–H_2O (4:1) at room temp. The CH_2Cl_2 extract was subjected to silica gel VLC with hexane containing increasing proportions of EtOAc. Further purification by VLC with hexane–$CHCl_3$–Me_2CO (5:3:2), EtOAc and a MeOH step-gradient was done to remove chlorophyll. Separation by MPLC with hexane–EtOAc–MeOH (16:4:1) and final purification by RP-HPLC with MeOH–H_2O (3:1) led to the isolation of compounds **1** (48.3 mg), **2** (5.0 mg), **3** (3.1 mg) and **4** (15.6 mg).

Macarangaflavanone A. 3'-Geranyl-4',5,7-trihydroxyflavanone (**1**). HREIMS *m/z* 408.1877 ($C_{25}H_{28}O_5$ requires 408.1929). EIMS *m/z* (rel. int.): 408 $[M]^+$ (< 1), 255 $[C_{18}H_{23}O]^+$ (< 1), 153 $[C_7H_5O_4]^+$ (32). $[\alpha]_D^{25}$ − 13.5 ($CHCl_3$; *c* 0.25). UV λ_{max}^{MeOH} nm (log ε): 286 (4.10), 320 (sh) (3.60); + NaOMe: 247 (4.12), 323 (4.32); + $AlCl_3$: 311 (4.23), 377 (3.41); + $AlCl_3$ + HCl: 310 (4.19), 377 (3.43); + NaOAc: 324 (4.29); + NaOAc + H_3BO_3: 288 (4.10). IR ν_{max}^{film} cm^{-1}: 3390 (OH); 2970, 2920 (C=CH); 1640 (conj. C=O); 1610, 1510, 1460 (ar. C=C). ^1H NMR: Table 1. ^{13}C NMR: Table 2.

Macarangaflavanone B. 4',5,7-Trihydroxy-3',6-bis(3-methyl-2-butenyl) flavanone (**2**). HREIMS *m/z* 408.1929 ($C_{25}H_{28}O_5$ requires 408.1929). EIMS *m/z* (rel. int.): 408 $[M]^+$ (45), 221 $[C_{12}H_{13}O_4]^+$ (21), 220 $[C_{12}H_{12}O_4]^+$ (25), 219 $[C_{12}H_{11}O_4]^+$ (20), 188 $[C_{13}H_{16}O]^+$ (21). $[\alpha]_D^{25}$ 0.0° (MeOH; *c* 0.5). UV λ_{max}^{MeOH} nm (log ε): 293 (4.25), 332 (sh) (3.59); + NaOMe: 247 (4.29), 330 (4.43); + $AlCl_3$: 295 (4.19); + $AlCl_3$ + HCl: 297 (4.15); + NaOAc: 330 (4.28); + NaOAc + H_3BO_3: 294 (4.21). IR ν_{max}^{film} cm^{-1}: 3360 (OH); 2970, 2920 (C=CH); 1640 (conj. C=O); 1600, 1500, 1450 (ar. C=C). 1H NMR: Table 1. ^{13}C NMR: Table 2.

Euchrestaflavanone A. 4',5,7-Trihydroxy-3',8-bis(3-methyl-2-butenyl) flavanone (**3**). EIMS identical with those of lespedezaflavanone B [8] and euchrestaflavanone A [6, 7]. $[\alpha]_D^{25}$ − 21.7 (MeOH; *c* 0.25), cf. − 35.0 [7], − 29.1 [8]. UV shifts agree with those of lespedezaflavanone B [8]. IR spectral data comparable with those of lespedezaflavanone B [8] and euchrestaflavanone A [6, 7]. ^1H NMR: Table 1. ^{13}C NMR: Table 2.

Bonannione A. 6-Geranyl-4',5,7-trihydroxyflavanone (**4**). EIMS in agreement with that of bonannione A [14]. $[\alpha]_D^{25}$ − 3.1 ($CHCl_3$; *c* 0.5), cf. − 2.6 [10]. UV λ_{max}^{MeOH} nm (log ε): 293 (4.26), 329 (sh) (3.61); + NaOMe: 246 (4.47), 329 (4.37); + $AlCl_3$: 295 (4.19); + $AlCl_3$ + HCl: 300 (4.16); + NaOAc: 329 (4.30); + NaOAc + H_3BO_3: 294 (4.22). IR ν_{max}^{film} cm^{-1}: 3350 (OH); 2920 (C=CH); 1640 (conj. C=O); 1600, 1520, 1490 (ar. C=C). 1H and ^{13}C NMR spectral data are consistent with those in ref. [10].

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REFERENCES

1. Henty, E. E. (1980) in *Harmful Plants in Papua New Guinea*, p. 61. Department of Forests, Division of Botany, Lae, Papua New Guinea.
2. Holdsworth, D. and Kerenga, K. (1987) *Int. J. Crude Drug Res.* **25**, 183.
3. Holdsworth, D. K. and N'Drawii, C. S. (1973) *Science in New Guinea* **1**, 11.
4. Holdsworth, D. K. (1974) *Science in New Guinea* **2**, 142.
5. Hnawia, E., Thoison, O., Guéritte-Voegelein, F. and Sévenet, T. (1990) *Phytochemistry* **29**, 2367.
6. Shirataki, Y., Komatsu, M., Yokoe, I. and Manaka, A. (1981) *Chem. Pharmacol. Bull.* **29**, 3033.
7. Shirataki, Y., Yokoe, I., Endo., M. and Komatsu, M. (1985) *Chem. Pharmacol. Bull.* **33**, 444.
8. Wang, M., Li, J. and Liu, W. (1987) *Phytochemistry* **26**, 1218.
9. Dawidar, A. M., Jakupovic, J., Abdel-Mogib, M. and Mashaly, I. A. (1994) *Phytochemistry* **36**, 803.
10. Bruno, M. and Savona, G. (1985) *Heterocycles* **23**, 1147.
11. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) in *The Systematic Identification of Flavonoids*. Springer-Verlag, Berlin.
12. Mitscher, L. A., Gollapudi, S. R., Khanna, I. K., Drake, S. D., Hanumaiah, T., Ramaswamy, T. and Rao, K. V. J. (1985) *Phytochemistry* **24**, 2885.
13. Bohm, B. A. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H., eds), p. 594. Chapman and Hall, London.
14. Fukai, T. and Nomura, T. (1990) *Heterocycles* **31**, 1861.
15. Sherif, E. A., Gupta, R. K. and Krishnamurti, M. (1980) *Tetrahedron Lett.* **21**, 641.
16. Yang, S. S., Gao, F., Mabry, T. J., Amer, M. E., Abdel-Kader, M. A., Mahmoud, Z. F. and Abdel-Salam, N. A. (1989) *Phytochemistry* **28**, 1749.

17. Wehrli, F. W. (1975) *J. C. S. Chem. Commun.* **1975**, 663.
18. Kalinowski, H.-O., Berger, S. and Braun, S. (1988) in *Carbon-13 NMR Spectroscopy*, p. 178. John Wiley and Sons, Chichester.
19. Shirataki, Y., Noguchi, M., Yokoe, I., Tomimori, T. and Komatsu, M. (1991) *Chem. Pharmacol. Bull.* **39**, 1568.
20. Orjala, J., Wright, A. D., Behrends, H., Folkers, G., Sticher, O. Rüegger, H. and Rali, T. (1994) *J. Nat. Prod.* **57**, 18.