Phytochemistry, Vol. 30, No. 9, pp. 3091–3093, 1991
Printed in Great Britain.

0031-9422/91 $3.00+0.00

COMPLEX FLAVONOIDS IN FARINOSE EXUDATE FROM *PITYROGRAMMA CALOMELANOS*

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(*Received* 30 *January* 1991)

Key Word Index—*Pityrogramma calomelanos*; Polypodiaceae; farinose exudate; complex flavonoid; calomelanols A–C.

Abstract—From the farinose exudate of *Pityrogramma calomelanos*, three new complex flavonoids named calomelanols A–C were isolated in addition to two known dihydrochalcones. The structures were characterized as 8-[3-(4-methoxyphenylpropionyl)]-5,7-dihydroxydihydroneoflavone for calomelanol A, 8-[3-(4-hydroxyphenylpropionyl)]-5,7-dihydroxydihydroneoflavone for calomelanol B, and 8-(3-phenylpropionyl)-5,7,4′-trihydroxydihydroneoflavone for calomelanol C, respectively, by spectroscopic evidence. The ^{13}C NMR assignment and the mass spectral fragmentation of the complex flavonoids are also described.

INTRODUCTION

Some plants such as *Primula* (Primulaceae) exude farina on their foliage. *Pityrogramma* species (Polypodiaceae) are also known to have a farinose exudate, and some of them are commonly called silverback or goldenback ferns according to differences in the farinose colour (white or yellow). In the chemical constituents of this exudate, the presence of a so-called complex flavonoid with a new C_6–C_3–C_6–C_3–C_6 skeleton, which corresponds to a compound fused by parts of flavone and neoflavone, was detected in addition to known flavoniods [1–3]; the structures of some of them were established by X-ray analysis [4], or unambiguous synthesis [5–7]. These complex flavonoid derivatives have been reported to play an important role in the chemosystematics of the genus *Pityrogramma* [8]. Wollenweber *et al.* pointed out the presence of other minor constituents of complex flavonoids, which have not been determined up to the present, in the farina of *P. calomelanos* [8]. To clarify the structures of the minor constituents and to contribute to the chemosystematics, the farinose exudate of *P. calomelanos* from Indonesia was examined.

RESULTS AND DISCUSSION

The farinose exudate of *P. calomelanos* obtained by rinsing with acetone was purified by silica gel chromatography leading to the isolation of complex flavonoids **1–4** in addition to two known dihydrochalcones.

Compound **1**, an amorphous powder, gave $[M]^+$ at *m/z* 388.1322 in the HR mass spectrum, the empirical formula corresponding to $C_{24}H_{20}O_5$ (calcd. 388.1311). The spectral data are superimposable on those of 8-(phenylpropionyl)-5,7-dihydroxydihydroneoflavone [1]. The mass spectrol fragmentation of the complex flavonoid is shown in Scheme 1 together with its prominent fragment ions **1a–f**. The assignment of the ^{13}C NMR spectrum supported by ^1H–^1H, ^1H–^{13}C, ^1H–^{13}C long range COSY and INEPT experiments is presented in Table 1.

Compound **2** also obtained as a amorphous powder gave $[M]^+$ at *m/z* 418.1437; its empirical formula is

Table 1. ^{13}C NMR spectral data of compounds **1–4**

	1	2	3	4
2	167.2 *s*	167.2 *s*	167.3 *s*	167.0 *s*
3	37.5 *t*	37.6 *t*	37.6 *t*	37.5 *t*
4	35.5 *d*	35.6 *d*	35.4 *d*	34.4 *d*
5	162.3 *s*	162.2 *s*	162.0 *s*	161.9 *s*
6	100.3 *d*	100.4 *d*	100.6 *d*	100.0 *d*
7	166.9 *s*	166.9 *s*	166.6 *s*	166.4 *s*
8	105.5 *s*	105.6 *s*	105.7 *s*	105.2 *s*
9	155.7 *s*	155.7 *s*	155.2 *s*	155.3 *s*
10	106.3 *s*	106.4 *s*	106.0 *s*	106.6 *s*
1′	142.8 *s*	142.9 *s*	142.8 *s*	133.1 *s*
2′,6′	128.1 *d*	128.2 *d*	128.1 *d*	128.9 *d*
3′,5′	129.6 *d*	130.1 *d*	130.0 *d*	116.5 *d*
4′	127.2 *d*	128.4 *d*	128.4 *d*	157.5 *s*
1″	142.8 *s*	134.6 *s*	134.1 *d*	142.5 *s*
2″,6″	129.9 *d*	130.8 *d*	130.3 *s*	129.3 *s*
3″,5″	130.1 *d*	115.1 *d*	116.0 *d*	129.6 *s*
4″	128.4 *d*	159.5 *s*	155.0 *s*	127.0 *d*
7″	31.9 *t*	31.6 *t*	31.5 *t*	31.6 *t*
8″	46.9 *t*	47.4 *t*	47.2 *t*	46.6 *t*
9″	205.5 *s*	205.8 *s*	206.0 *s*	205.2 *s*
OMe	—	55.8 *q*	—	—

Measured in d_6-acetone. All signals of **1** and **2** were assigned using ^1H–^{13}C long range COSY, and those of **3** and **4** were assigned on the basis of the results of **1** and **2**. Multiplicities were determined by INEPT.

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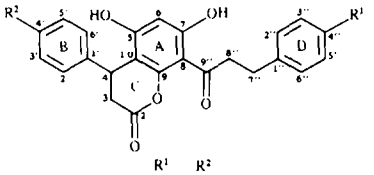
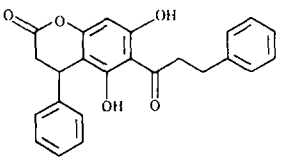


	R^1	R^2
1	H	H
2	OMe	H
3	OH	H
4	H	OH



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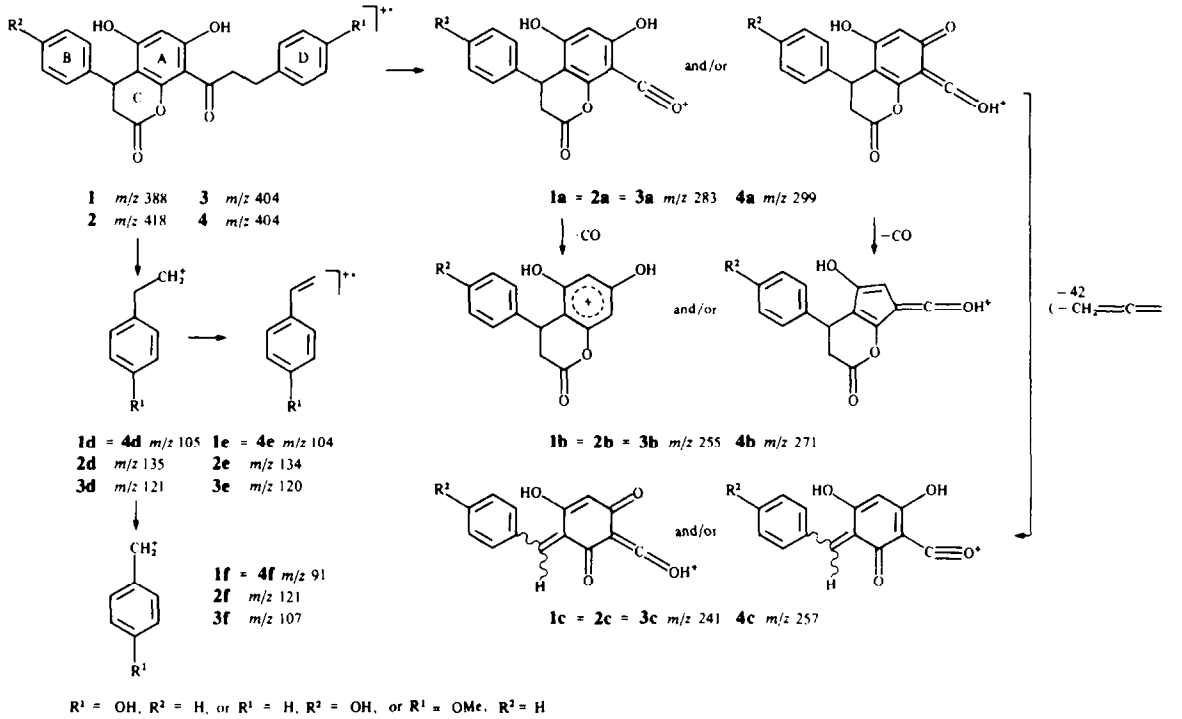
$C_{25}H_{22}O_6$ (calcd. 418.1416). In the ^1H NMR spectrum, the presence of a methoxyl group (δ3.75) and a *para*-substituted benzene ring [δ6.81 and 7.25 (2H, each *s*, J = 8.8 Hz)] was confirmed. In the EI mass spectrum, the significant fragment ions at *m/z* 283 (**2a**), 255 (**2b**), 241 (**2c**) corresponded to **1a–c** of compound **1**, respectively, and those at *m/z* 135 (**2d**), 134 (**2e**) and 121 (**2f**) to **1d–f**, indicating that the methoxyl group is substituted on the D ring. Compound **1** and its isomer (**5**) [8] showed different results towards the Gibbs test, that is, **5** reacted immediately to turn blue, but **1** did so only very slowly. The Gibbs test to **2** showed a delayed colouration like that of **1**, which indicated a partial structure based on the substitution of a phenylpropionyl group the same as **1**. The ^{13}C NMR spectral data also supported that the position of the phenylpropionyl group was at C-8 of a dihydroneoflavone moiety. The structure of **2** is thus, 8-[3-(4-*p*-methoxyphenyl)propionyl]-5,7-dihydroxydihydroneoflavone and named calomelanol A.

Compounds **3** and **4** could be separated by TLC using benzene–ethylacetate and gave respective $[M]^+$ at *m/z* 404.1266 and 404.1233 in the HR mas spectrum, both of which correspond to $C_{24}H_{20}O_6$ (calcd. 404.1260). The ^1H NMR spectra of **3** and **4** were fundamentally identical with a complex flavonoid skeleton, and the structures were regarded as not 6-(3-phenylpropionyl)-, but 8-(3-phenylpropionyl)-dihydroneoflavone from their behaviour in the Gibbs test which was the same as **1** and **2**. A set of doublet (A_2B_2) at δ6.60 and 6.99 (2H, each *d*, J = 9 Hz) in **3**, and at δ6.75 and 7.01 (2H, each *d*, J = 9 Hz) in **4** showed that both **3** and **4** have a *para*-substituted benzene ring. The location of hydroxyl groups was determined as follows. In the case of **3**, fragment ions based on rings A, B and C were observed at *m/z* 283 (**3a**), 255 (**3b**) and 241 (**3c**), as in **1** and **2**. Such ions in **4** were at *m/z* 299 (**4a**), 271 (**4b**) and 257 (**4c**). On the other hand, fragment ions based on the D ring of **4** were 105 (**4d**), 104 (**4e**) and 91 (**4f**) as in **1**, while the fragments of **3** were at *m/z* 121 (**3d**), 120 (**3e**) and 107 (**3f**). These results indicated that the position of the hydroxyl group was at C-4 of a dihydrochalcone moiety (ring D) in **3**, and at C-4 of a dihydroneoflavone moiety (ring B) in **4**. The structures of **3** and **4** were, therefore, determined to be 8-[3-(*p*-hydroxyphenylpoionyl)]-5,7-dihydroxydihydroneoflavone and 8-(3-phenyl-pro-



Scheme 1. Proposed mass spectral fragmentation of compounds **1–4**.

pionyl)-5,7,4′-trihydroxydihydroneoflavone, respectively. These new complex flavonoids were named calomelanol B for **3**, and calomelanol C for **4**. The structure of **3** was further confirmed by synthesis. 5,7-Dihydroxy-8-(*p*-coumaroyl)dihydroneoflavone (T-2) [5] was hydrogenated by Pd/C under a hydrogen atomosphere to give **3**. The properties of the prepared compoud agreed well with those of the natural product **3**. It is noteworthy that **4** is the first instance of a complex flavonoid possessing oxygen-function on the B ring.

In addition to above four compounds, 2′,6′-dihydroxy-4′-methoxydihydrochalcone and 2′,6′-dihydroxy-4′,4′-dimethoxydihydrochalcone were also isolated and their structures determined from their spectroscopic data.

EXPERIMENTAL

Plant material. Fronds of *P. calomelanos* were collected at Busaki Bali in Indonesia in July, 1990. Voucher specimens are deposited at the Herbarium of Gifu Pharmaceutical University.

Extraction and isolation of compounds **1–4**. Dried fronds (2.75 kg) were rinsed with Me_2CO (15 l) and the extract concd to give a residue (90 g) which was subjected to silica gel CC and elution with hexane–EtOAc (3:1). From early frs, 2′,6′-dihydroxy-4′-methoxydihydrochalcone (3.7 g) and 2′,6′-dihydroxy-4,4′-dimethoxydihydrochalcone (2.8 g) were crystallized. Frs containing **1–4** monitored by TLC were further chromatographed on silica gel CC, and finally purified by prep. TLC (C_6H_6–EtOAc, 5:1) to give **1** (1.3 g), **2** (250 mg), **3** (45 mg) and **4** (32 mg), respectively.

Compound **1**. Amorphous powder. EIMS *m/z* (%): 388 ([M]$^+$, 44), 283 (100), 255 (58), 241 (62), 203 (11), 105 (13), 91 (167), 104 (16), 77 (20). UV λ^{MeOH} nm: 285, 327. ^{1}H NMR (270 MHz, $CDCl_3$) δ: 3.05–3.52 (6H, *m*, H-3, H-7″, H-8″), 4.53 (1H, *dd*, *J* = 6, 3 Hz, H-4), 6.18 (1H, *s*, H-6), 7.10–7.31 (10H, *m*, H-2′, H-6′ and H-2″, H-6″), 13.62 (1H, *s*, OH-7).

Compound **2** (*calomelanol A*). Amorphous powder. EIMS *m/z* (%): 418 ([M]$^+$, 20), 400 (4), 383 (18), 255 (3), 241 (20), 135 (13), 134 (71), 121 (100), 107 (2). UV λ^{MeOH} nm: 283, 323. ^{1}H NMR (270 MHz, Me_2CO) δ: 2.95 (2H, *m*, H-7″), 3.03 (1H, *dd*, *J* = 16, 2 Hz, H-3), 3.28 (1H, *dd*, *J* = 16, 7 Hz, H-3), 3.45 (2H, *m*, H-8″), 3.75 (3H, *s*, OMe), 4.70 (1H, *dd*, *J* = 7, 2 Hz, H-4), 6.26 (1H, *s*, H-6), 6.81 (2H, *d*, *J* = 8.8 Hz, H-3″, H-5″), 7.25 (2H, *d*, *J* = 8.8 Hz, H-2″, H-6″), 7.18–7.31 (5H, *m*, H-2′, H-6′), 13.55 (1H, *s*, OH-7).

Compound **3** (*calomelanol B*). Amorphous powder. EIMS *m/z* (%): 404 ([M]$^+$, 33), 283 (36), 256 (11), 255 (8), 249 (29), 121 (13), 120 (100), 107 (40). UV λ^{MeOH} nm: 283, 323. ^{1}H NMR (270 MHz, d_6-Me_2CO) δ: 2.77 (2H, *m*, H-7″), 2.87 (1H, *dd*, *J* = 14, 2 Hz, H-3), 3.15 (1H, *dd*, *J* = 14, 7 Hz, H-3), 3.28 (2H, *m*, H-8″), 4.56 (1H, *dd*, *J* = 7, 2 Hz, H-4), 6.15 (1H, *s*, H-6), 6.60 (2H, *d*, *J* = 9 Hz, H-3″, H-5″), 6.99 (2H, *d*, *J* = 9 Hz, H-2″, H-6″), 7.12 (5H, *m*, H-2′–H-6′), 13.44 (1H, *s*, OH-7).

Compound **4** (*calomelanol C*). Amorphous powder. EIMS *m/z* (%): 404 ([M]$^+$, 100), 299 (84), 257 (62), 272 (67), 271 (16), 205 (93), 105 (11), 104 (16), 91 (78). UV λ^{MeOH} nm: 284, 325. ^{1}H NMR (270 MHz, d_6-Me_2CO) δ: 2.95 (1H, *dd*, *J* = 13, 3 Hz, H-3), 3.03 (2H, *m*, H-7″), 3.23 (1H, *dd*, *J* = 13, 7 Hz, H-3), 3.48 (2H, *m*, H-8″), 4.60 (1H, *dd*, *J* = 7, 3 Hz, H-4), 6.27 (1H, *s*, H-6), 6.75 (2H, *d*, *J* = 9 Hz, H-3′, H-5′), 7.01 (2H, *d*, *J* = 9 Hz, H-2′, H-6′), 7.22 (5H, *m*, H-2″–H-6″), 13.52 (1H, *s*, OH-7).

Synthesis of compound **3**. An EtOAc soln containing 5,7-dihydroxy-8-(*p*-coumaroyl)-dihydroneoflavone (12 mg) and 10% Pd/C (2 mg) was stirred at room temp. under a H_2 atmosphere for 4 hr. After filtration of the reaction mixt., the filtrate was concd under red. pres. to give **3** 10 mg) as an amorphous powder. The mass and ^{1}H NMR spectral data were coincident with those of **3**.

REFERENCES

1. Wagner, H., Seligmann, O., Chari, M. V., Wollenweber, E., Dietz, V. H., Donnelly, D. M. X., Meegan, M. J. and O'Donnell, B. (1979) *Tetrahedron Letters* 4296.
2. Favre-Bonvin, J., Jay, M., Wollenweber, E. and Dietz. V. H. (1980) *Phytochemistry* **19**, 2043.
3. Dietz, V. H., Wollenweber, E., Favre-Bonvin, J. and Gomez, L. D. (1980) *Z. Naurforsch.* **35c**, 36.
4. Donnelly, D. M. X., Fukuda, N., Wollenweber, E., Polonsky, J. and Prange, T. (1987) *Phytochemistry* **26**, 1143.
5. Iinuma, M., Mstsuura, S. and Asai, F. (1983) *Heterocycles* **20**, 1923.
6. Iinuma, M., Hamada, K., Mizuno, M., Asai, F. and Wollenweber, E. (1986) *Z. Naturforsch.* **41c**, 681.
7. Asai, F., Iinuma, M., Tanaka, T. and Mizuno, M. (1990) *Chem. Pharm. Bull.* **38**, 1079.
8. Wollenweber, E. and Dietz, V. H. (1980) *Biochem. System. Ecol.* **8**, 21.