TWO COMPLEX FLAVONOIDS IN THE FARINOSE EXUDATE OF *PITYROGRAMMA CALOMELANOS*

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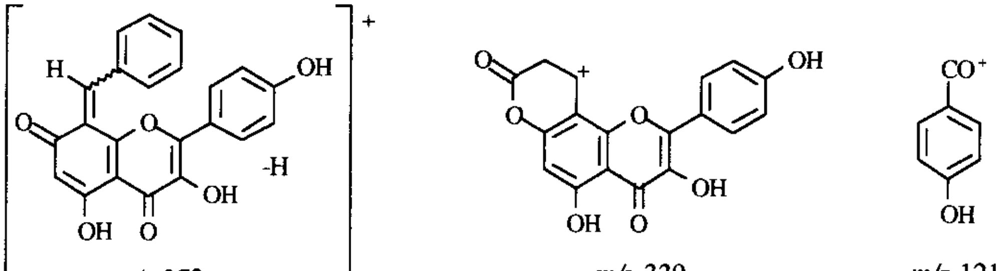
Abstract ----- From the farinose exudate of *Pityrogramma calomelanos,* two new complex flavonoids named calomelanols D and E were isolated. Their structures were determined by spectroscopic analysis.

Pityrogramma species (Polypodiaceae) occasionally spout farinose on the surface of frond, and some of them are commonly called as "Gold Fern" or "Silver Fern" by the difference of farinose color.[1] Several new compounds with a novel C6-C3-C6-C3-C6 skeleton (complex flavonoids) were isolated in addition to simple chalcones or dihydrocalcones. The complex flavonoid is regarded as a flavonoid condensed with A neoflavonoid *via* common phloroglucinol moiety (A ring). Among them, eight complex flavonoids, D-1, D-2a and D-2b in *P. calomelanos*,[2] T-1, T-2 and T-3 in *P. trifoliata*,[3] and X-1 and X-2 in *P. calomelanos* var. *aureoflava*[4] were isolated and their structures were determined, and some of them were confirmed by synthesis.[5-7] Besides these flavones, the presence of minor cognates in the farinose exudate of *P. calomelanos* has been suggested.[8] To clarify the structures of them, the farinose exudate of *P. calomelanos* in Indonesia was examined. In succession to our preceding paper[9] reporting three new 8-(3-phenylpropionyl)dihydrochalcones, named calomelanols A, B and C, the isolation and structure determination of other two new complex flavonoids are described in this paper.

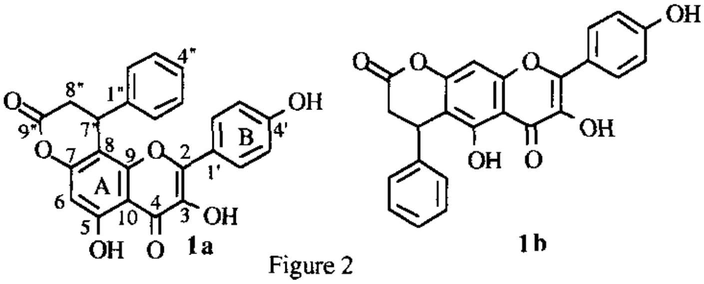
Farinose exudate of *P. calomelanos* was rinsed with acetone, and the concentrated extract was purified with silica gel chromatography to lead the isolation of two compounds.

Compound (**1**), calomelanol D, yellow needles, gave M$^+$ at *m/z* 416.0889 in the hrms and the empirical formula corresponds to $C_{24}H_{16}O_7$. The uv spectral data suggested that **1** has a flavonol core, and the behaviors caused by addition of shift reagents were closely similar to those of kaempferol.[10] In the ^{1}H-nmr spectrum, a set of three-proton double doublets at δ 3.05 (*J*= 14, 2 Hz), 3.43 (*J*= 14, 7 Hz) and 5.12 (*J*= 7, 2 Hz) indicated the presence of a partial structure of $PhCHCH_2CO$. Furthermore, a set of two-proton doublets [δ 7.00 and 8.01 (each *J*= 9 Hz)], an aromatic proton singlet (δ 6.56), and five-proton multiplet which is assigned to a mono-substituted benzene ring, and three hydroxyl groups [δ 8.34, 9.06 and 12.30 (chelated)] were observed. In the eims, prominent fragment peaks were observed at *m/z* 373, 339 and 121 (Figure 1). These results showed that the partial structure, $PhCHCH_2CO$, was fused with a kaempferol skeleton on the A ring. The behavior of uv and the appearance of a chelated hydroxyl group in ^{1}H-nmr spectra permitted two possible structures (**1a**) or (**1b**) (Figure 2). The confirmation of structure for **1** was depended on 2D nmr experiments. That is, in the ^{1}H-^{13}C COSY (Figure 3), an aromatic singlet proton caused a cross peak with a carbon signal at δ 99.8. In the long range ^{1}H-^{13}C COSY (Figure 3), the carbon at δ 99.8 also caused a cross peak with a chelated hydroxyl group. Consequently, **1a** was preferable to **1b** as a structure of calomelanol D. The stereochemisty of C-7" is now being investigated.



m/z 373 *m/z* 339 *m/z* 121

Figure 1



1a **1b**

Figure 2

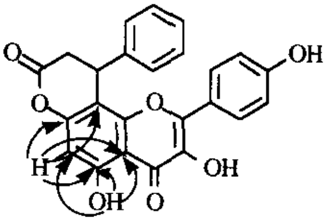
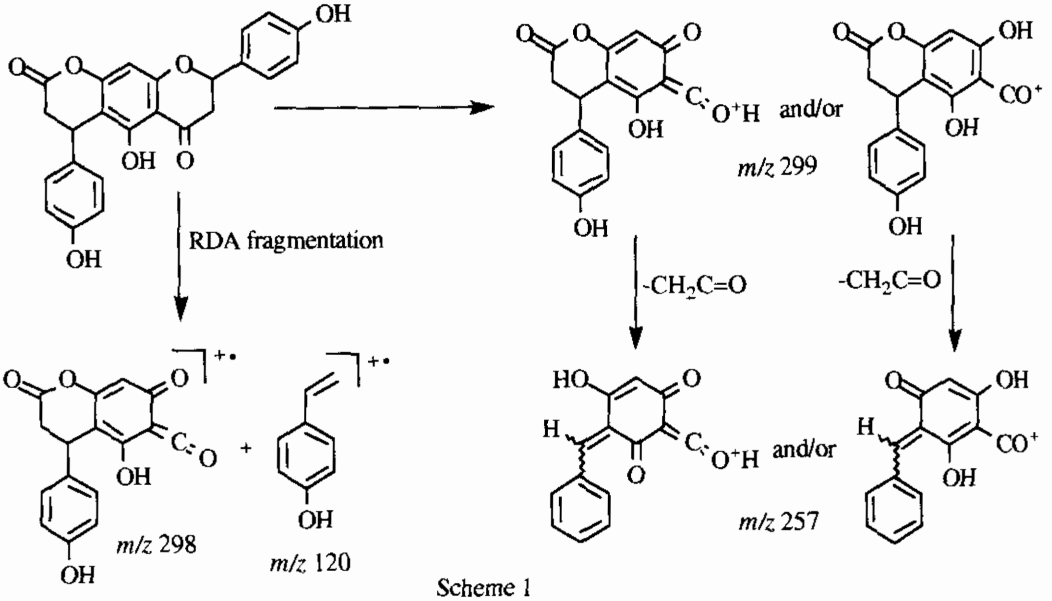


Figure 3

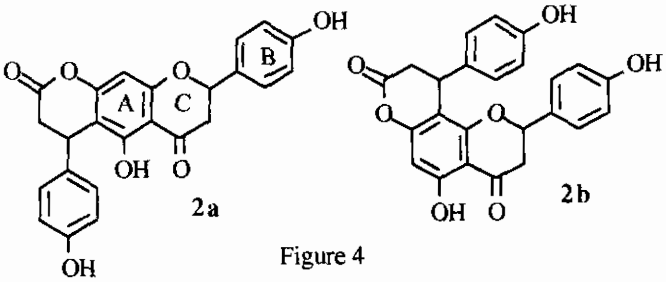
Compound (**2**), calomelanol E, a colorless powder, gave M$^+$ *m/z* 418.1083 in the hrms, and the empirical formula is $C_{24}H_{18}O_7$. In the ^{1}H-nmr spectrum, two sets of three-proton double doublets were observed [δ

2.89 (J= 17, 2 Hz), 3.25 (J= 17, 6 Hz), 4.48 (J= 6, 2 Hz); δ 2.81 (J= 17, 3 Hz), 3.39 (J= 17, 13 Hz), 5.61 (J= 13, 3 Hz)]. The former set suggested the presence of a partial structure of PhCHCH2CO. The latter was assigned to H-3 and H-2 of a flavanone skeleton. Furthermore, the spectrum also showed the presence of two *p*-substituted benzene rings [δ 6.71, 6.92 (each J= 8 Hz) and 6.82, 7.35 (each J= 9 Hz)], an aromatic proton (δ 6.37) and three hydroxyl groups [δ 9.40, 9.65 and 12.41 (chelated)]. In the eims, significant fragments were appeared at *m/z* 299, 298, 257 and 120. The fragment ions could be drawn as in Scheme 1.



Scheme 1

These spectral data mentioned above suggested that the structure of **2** was either **2a** or **2b** shown in Figure 4. In the ^{1}H-^{13}C COSY, an aromatic proton based on the A ring caused a cross peak with a carbon at δ 96.1. In the long range ^{1}H-^{13}C long COSY, a chelated hydroxyl group caused cross peaks with three quaternary carbons assigned to C-5 (δ 159.0), C-6 (δ 105.9) and C-10 (δ 104.8) (Figure 5). Consequently, the structure of calomelanol E was determined to be **2a**.



2a **2b**

Figure 4

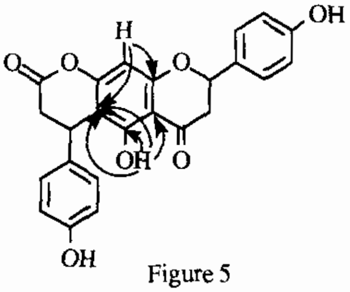


Figure 5

EXPERIMENTAL

Plant material and extraction were described in preceding paper.[8]

Isolation of compounds 1 and 2. A more polar fraction containing D-1 and calomelanols A-C was purified by silica gel column chromatography (eluent: *n*-hexane-acetone = 3:1 and C_6H_6-EtOAc = 5:1) and recrystalization to give **1** (45 mg) and **2** (60 mg) in pure form.

Compound 1 (calomelanol D): mp 235 °C (dec.) (MeOH); yellow needles; $[\alpha]_D$ -20° (c 0.06, MeOH); ir (KBr)1760, 1660, 1630, 1618 cm^{-1}; hrms *m/z* 416.0889 (Calcd 416.0869 for $C_{24}H_{16}O_7$); eims *m/z* (rel. int.): 416 (M$^+$, 100), 388 (18), 373 (38), 339 (22), 241 (1). 239 (7), 208 (4), 205 (7), 184 (11), 121 (24); uv (nm, MeOH): 270, 325sh, 373, +NaOMe: 283, 325, 418, +$AlCl_3$: 265, 295sh, 360, 428; ^{1}H-nmr (acetone-d_6) δ: 3.05 (1H, dd, *J*= 14, 2 Hz, H-8"), 3.43 (1H, dd, *J*= 14, 7 Hz, H-8"), 5.12 (1H, br dd, *J*= 7, 2 Hz, H-7"), 6.56 (1H, s, H-6), 7.00 (2H, d, *J*= 9 Hz, H-3' and 5'), 7.20-7.28 (5H, m, H-2"-6"), 8.01 (2H, d, *J*= 9 Hz, H-2' and 6'), 8.34, 9.06 (1H each, br s, OH), 12.30 (1H, s, C_5-OH); ^{13}C-nmr (acetone-d_6): all carbons were assigned by the aids of C-H and long range C-H COSY) δ: 148.2 (C-2), 137.6 (C-3), 177.1 (C-4), 161.4 (C-5), 99.8 (C-6), 158.0 (C-7), 105.1 (C-8), 153.3 (C-9), 107.4 (C-10), 123.1 (C-1'), 130.7 (C-2' and 6'), 116.6 (C-3' and 5'), 160.6 (C-4'), 142.5 (C-1"), 127.7 (C-2" and 6"), 130.1 (C-3" and 5"), 128.3 (C-4"), 35.8 (C-7"), 37.7 (C-8"), 166.0 (C-9").

Compound 2 (calomelanol E): mp 265 °C (dec.) (MeOH); a colorless powder ,$[\alpha]_D$ -51° (c 0.06, MeOH); ir (KBr): 1760, 1640, 1590 cm^{-1},hrms *m/z* 418.1083 (Calcd 418.1053 for $C_{24}H_{18}O_7$); eims *m/z* (rel. int.): 418 (M$^+$, 100), 402 (20), 390 (9), 375 (7), 325 (9), 324 (8), 323 (7), 299 (22), 298 (18), 297 (15), 283 (9), 280 (29), 270 (29), 257 (11), 255 (20), 252 (33), 205 (38), 147 (11), 120 (53), 107 (22); uv (nm, MeOH): 280, 340, +NaOMe: 285sh, 332, +$AlCl_3$: 280, 340; ^{1}H-nmr (acetone-d_6) δ: 2.92 (4H, m, H-3 and 8"), 4.59 (1H, br dd, *J*= 6, 2 Hz, H-7"), 5.62 (1H, dd, *J*= 13, 3 Hz, H-2), 6.27 (1H, s, H-8), 6.77 (2H, d, *J*= 8 Hz, H-3" and 5'), 6.92 (2H, d, *J*= 9 Hz, H-3' and 5'), 7.02 (2H, d, *J*= 8 Hz, H-2" and 6"), 7.43 (2H, d, *J*= 9 Hz, H-2' and 6'), 8.30, 8.58 (1H each, br s, OH), 12.49 (1H, s, C_5-OH); (DMSO-d_6)(assigned by the H-H COSY) δ: 2.81 (1H, dd, *J*= 17, 3 Hz, H-3*eq*), 2.89 (1H, dd, *J*= 17, 2 Hz, H-8"), 3.25 (1H, dd, *J*= 17, 6 Hz, H-8"), 3.39 (1H, dd *J*= 17, 13 Hz, H-3*ax*), 4.48 (1H, br dd, *J*= 6, 2 Hz, H-7"), 5.61 (1H, dd, *J*= 13, 3 Hz, H-2), 6.37 (1H, s, H-8), 6.71 (1H, d, *J*= 8 Hz, H-3" and 5"), 6.82 (2H, d, *J*= 9 Hz, H-3' and 5'), 6.92 (2H, d, *J*= 8 Hz, H-2" and 6"), 7.35 (2H, d, *J*= 9 Hz, H-2' and 6'), 9.40 (1H, br s, C4"-OH), 9.65 (1H, br s, C4'-OH), 12.41 (1H, s, C_5-OH); ^{13}C-nmr (DMSO-d_6): all carbons were assigned by the aids of C-H and long range C-H COSY) δ: 78.7 (C-2), 41.9 (C-3), 198.2 (C-4), 159.0 (C-5), 105.9 (C-6), 158.4 (C-7), 96.1 (C-8), 161.4 (C-9), 104.8 (C-10), 128.3 (C-1'), 128.5 (C-2' and 6'), 115.2 (C-3' and 5'), 157.9 (C-4'), 131.2 (C-1"), 127.5 (C-2" and 6"), 115.5 (C-3" and 5"), 156.4 (C-4"), 32.4 (C-7"), 36.7 (C-8"), 166.7 (C-9").

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