# Synthesis and Structure Confirmation of the Complex Flavonoids in *Pityrogramma calomelanos*

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**By unambiguous synthesis, the structures of two complex flavonoids in the farinose exudate of *Pityrogramma calomelanos* were established to be 8-(3-phenylpropionyl)-5,7-dihydroxyneoflavanone [8-(3-phenylpropionyl)-5,7-dihydroxy-4-phenylchroman-2-one] (1) and 8-(2-carboxy-1-phenylethyl)-5,7-dihydroxyflavone δ-lactone (3).**

**Keywords** *Pityrogramma calomelanos*; complex flavonoid; synthesis; 8-(3-phenylpropionyl)-5,7-dihydroxyneoflavanone; 8-(2-carboxy-1-phenylethyl)-5,7-dihydroxyflavone δ-lactone

From the farinose exudate of *Pityrogramma* species (Gymnogrammoideae), several compounds with a new $C_6$–$C_3$–$C_6$–$C_3$–$C_6$ skeleton (complex flavonoids) were isolated,[1-3] and some of their structures have been established by X-ray analysis[4] or unambiguous synthesis.[5,6] These compounds have been reported to play an important role in the chemotaxonomy of the genus *Pityrogramma*.[7] A complex flavonoid can be regarded as a flavonoid condensed with a neoflavonoid *via* a common phloroglucinol moiety. A characteristic feature of the structure is that a $C_6$–$C_3$ moiety is located exclusively at the C-8 position of a flavone nucleus (Chart 1). In the present paper, confirmation of the structures **1** and **3** is described.

Compounds **1** and **3** were reported as constituents of the exudate of *Pityrogramma calomelanos*,[1] together with a flavonol derivative of **3** (Chart 2). Among them, **3** and its cognate have not been separated completely, but their structures were both deduced by means of mass spectrometry (MS).[1] The synthesis of **1** [8-(3-phenylpropionyl)-5,7-dihydroxyneoflavanone or 8-(3-phenylpropionyl)-5,7-dihydroxy-4-phenylchroman-2-one] and its isomer **2** [6-(3-phenylpropionyl)-5,7-dihydroxyneoflavanone] will be described first. Usual benzylation of a mixture of 8-acetyl-5,7-dihydroxy- and 6-acetyl-5,7-dihydroxyneoflavone prepared by the Pechmann condensation of phloroacetophenone with ethyl benzoylacetate[5] afforded a mixture of the respective 5,7-dibenzyl ethers, which was fractionated by chromatography to give 8-acetyl-5,7-dibenzyloxyneoflavone (**5**) and 6-acetyl-5,7-dibenzyloxyneoflavone (**6**). The structures were supported by the chemical shifts of their protons (**5**: 2.59 ppm, **6**: 2.40 ppm) and aromatic protons (**5**: 6.41 ppm assigned to H-6, **6**: 6.86 ppm to H-8) in the proton nuclear magnetic resonance ($^1$H-NMR) spectra.[8] Condensation of **5** with benzaldehyde in the presence of potassium hydroxide gave 4′,6′-dibenzyloxy-3′-(2-carboxy-1-phenylethenyl)-2′-hydroxychalcone (**7**) *via* ring cleavage of the lactone moiety. The chalcone was lactonized into the corresponding neoflavone (**8**) on treatment with acetic anhydride and fused sodium acetate in benzene. Hydrogenation accompanied by debenzylation of **8** at atmospheric pressure over Pd–C gave the desired neoflavanone **1** (mp 162—163 °C) (lit.[1] mp 168 °C). Compound **2** (mp 170—171 °C) was also obtained similarly. Comparison of the spectral properties of **1** and **2** with those of the natural neoflavanone (D-1)[1] indicated that the neoflavanone (D-1) was identical with 8-(3-phenylpropionyl)-5,7-dihydroxyneoflavanone (**1**).

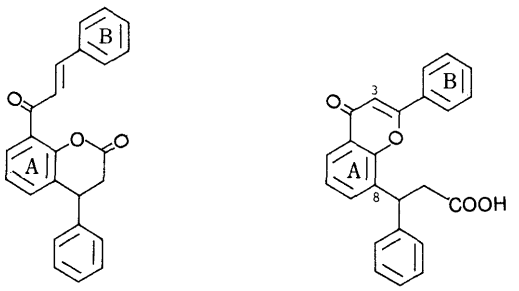
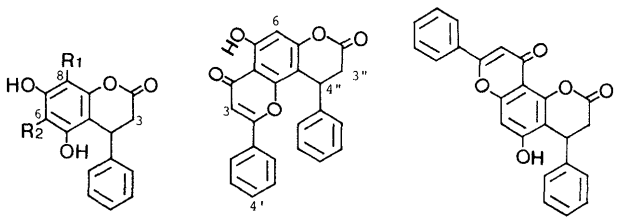



Chart 1




3 4

1 : $R_1$ = , $R_2$ = H

2 : $R_1$ = H , $R_2$ =

Chart 2

On the other hand, another complex flavonoid in *P. calomelanos* was proposed to be either **3** or **4**.[1] The structure **3** is preferred to **4** on biosynthetic grounds, that is, a $C_3$–$C_6$ moiety forming a neoflavanone skeleton is located at C-8 of the flavone nucleus in **3**, but at C-6 in **4**. The mode of C–C linkage is identical with that in the complex flavonoids in *P. calomelanos* var. *aureoflava*, the structures of which have been established. Therefore a synthesis of **3** was planned. 3′-(2-Carboxy-1-phenylethyl)-2′-hydroxy-4′,6′-diisopropoxychalcone[5] was esterified with 2-methoxyethanol in the presence of phosphoric acid, and the resulting ester was oxidized with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) to give two flavone derivatives **9** [*m/z* (rel. int.) 544 ($M^+$, trace), 529 ($M^+ - 15$, 100)] and **10** [*m/z* (rel. int.) 542 ($M^+$, 73), 527 ($M^+ - 15$, 100)]. Compound **10** was hydrogenated to **9**. Compounds **9** and **10** were determined to be the methoxyethyl esters of 8-(2-carboxy-1-phenylethyl)-5,7-diisopropoxy- and 8-(2-carboxy-1-phenylethenyl)-5,7-diisopropoxyflavone, respectively. Hydrolysis of **9** following deisopropylation with boron trichloride gave 8-(2-carboxy-1-phenylethyl)-5,7-dihydroxyflavone (**11**), which was lactonized to give **3**. The spectral prop-

erties of **3** agreed well with those of the natural product (D-2a).[1,6] Direct comparison of **3** with D-2a on thin layer chromatography (TLC) also showed good coincidence.[9] Therefore the structure of the other complex flavonoid was confirmed to be 8-(2-carboxy-1-phenylethyl)-5,7-dihydroxyflavone $\delta$-lactone.

Thus, the structures of the complex flavonoids in *Pityrogramma* species were confirmed by synthesis.

## Experimental

Melting points were determined on a Büchi melting point apparatus and are uncorrected. Electron ionization mass spectra (EIMS) were taken on a JEOL-D300 machine operating at 70 eV with a direct inlet system. Ultraviolet (UV) spectra were recorded on a Hitachi 323 spectrometer. $^1$H-NMR spectra were recorded by using tetramethylsilane as an internal standard on Hitachi R-20B and JNM-GX-270 spectrometers at 60 and 270 MHz. Chemical shifts are quoted in parts per million ($\delta$).

**Benzylation of a Mixture of 8- and 6-Acetyl-5,7-dihydroxyneoflavone** An *N,N*-dimethylformamide (DMF) solution (100 ml) containing a mixture of the two neoflavones (6.5 g, 22 mmol), benzyl chloride (9.8 g, 77 mmol) and potasium carbonate (15.2 g, 110 mmol) was heated with stirring at 120 °C for 5 h. The mixture was poured into 1 N HCl after cooling, and the acidified solution was extracted with EtOAc. The EtOAc layer was evaporated and the residue was subjected to silica gel column chromatography with $CHCl_3$. Two 5,7-dibenzylated neoflavones **5** in the later fraction (2.7 g) and **6** in the earlier fraction (2.4 g) were obtained. **5**: mp 166—167 °C (EtOAc–$C_6H_{14}$), yellow rectangles. EIMS *m/z* (rel. int.): 476 ($M^+$, 44), 458 (8), 434 (18), 386 (21), 385 (25), 371 (66), 343 (32), 309 (7), 281 (7), 181 (42), 91 (100). $^1$H-NMR ($CDCl_3$, 60 MHz) $\delta$: 2.59 (3H, s, $COCH_3$), 4.71, 5.14 (2H, each s, $OC\underline{H}_2Ph$), 5.96 (1H, s, H-3), 6.41 (1H, s, H-6), 6.70—6.94 (5H, m, side phenyl), 7.18, 7.34 (5H, each s, $OCH_2\underline{Ph}$). **6**: mp 161—162 °C (EtOAc–$C_6H_{14}$), pale yellow needles. EIMS *m/z* (rel. int.): 476 ($M^+$, 27), 458 (15), 434 (20), 386 (22), 385 (45), 371 (18), 368 (40), 343 (45), 281 (6), 181 (40), 91 (100). $^1$H-NMR ($CDCl_3$, 60 MHz) $\delta$: 2.40 (3H, s, $COCH_3$), 4.32, 5.22 (2H, s, $OC\underline{H}_2Ph$), 6.17 (1H, s, H-3), 6.86 (1H, s, H-8), 7.15—7.38 (5H, m, side phenyl), 7.41 (10H, s, 2 × $OCH_2\underline{Ph}$).

**Synthesis of 8-(3-Phenylpropionyl)-5,7-dihydroxyneoflavanone (1)** An 80% ethanol solution (30 ml) containing **5** (2.2 g, 4.6 mmol), benzaldehyde (490 mg, 4.6 mmol) and KOH (3 g) was stirred overnight at room temperature. The mixture was poured into 2 N HCl, and the yellow mass that precipitated was recrystallized from EtOH to give **7** as orange-yellow needles (1.3 g), mp 202—204 °C. EIMS *m/z* (rel. int.): 564 ($M^+$, 3), 538 (9), 473 (30), 447 (100), 384 (10), 357 (13), 343 (87), 253 (17), 193 (10), 131 (65), 103 (27), 91 (100). $^1$H-NMR ($CDCl_3$, 270 MHz) $\delta$: 4.71, 5.14 (2H, each s, $OC\underline{H}_2Ph$), 6.00 (1H, s, C=CHCO), 6.41 (1H, s, H-6), 6.71—7.50 (22H, m, 4 × Ph, COCH=CH). A dried benzene solution (50 ml) containing **7** (750 mg), $Ac_2O$ (2 ml) and fused AcONa (1 g) was refluxed for 4 h and poured into water. The solution was stirred for 3 h. A ring-closed compound (**8**, 540 mg) was obtained from an EtOAc extract of the solution as pale yellow needles, mp 201—202 °C (EtOAc–$C_6H_{14}$). EIMS *m/z* (rel. int.): 564 ($M^+$, 13), 473 (82), 445 (31), 384 (23), 371 (9), 355 (7), 281 (6), 279 (5), 193 (27), 180 (8), 131 (66), 103 (40), 91 (100). $^1$H-NMR ($CDCl_3$, 270 MHz) $\delta$: 4.70, 5.13 (2H, each s, $OC\underline{H}_2Ph$), 5.99 (1H, s, H-3), 6.38 (1H, s, H-6), 6.78—7.55 (20H, m, 4 × Ph), 7.07 (1H, d, $J$=16.1 Hz, COCH=C$\underline{H}$), 7.41 (1H, d, $J$=16.1 Hz, COCH=). An EtOAc solution (50 ml) containing **8** (280 mg) and 10% Pd–C (120 mg) was stirred under a hydrogen atmosphere for 5 h. By the usual work-up of the reaction mixture, **1** (75 mg) was obtained as colorless needles, mp 162—163 °C ($C_6H_6$–$C_6H_{14}$) (lit.[1] mp 168 °C). EIMS *m/z* (rel. int.): 388 ($M^+$, 68), 370 (11), 360 (4), 358 (6), 327 (5), 298 (6), 283 (100), 269 (80), 265 (73), 241 (64), 213 (15), 179 (11), 131 (12), 115 (9), 104 (14), 91 (43). $^1$H-NMR (DMSO-$d_6$, 270 MHz) $\delta$: 2.89 (1H, dd, $J$=15.8, 1.5 Hz, H-3 *cis*), 2.94 (2H, t, $J$=7.4 Hz, $COCH_2C\underline{H}_2$), 3.27 (1H, dd, $J$=15.8, 7.1 Hz, H-3 *trans*), 3.37 (2H, t, $J$=7.4 Hz, $COCH_2$), 4.52 (1H, dd, $J$=7.1, 1.5 Hz, H-4), 6.24 (1H, s, H-6), 7.09—7.35 (10H, m, 2 × Ph), 11.00 (1H, br s, $C_7$-OH), 12.92 (1H, s, $C_5$-OH). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 237 (4.15), 285 (4.08), 325sh (3.59). +NaOMe: 249sh, 329. +$AlCl_3$: 238, 311, 364. +$AlCl_3$/HCl: 237, 285sh, 308, 360. +NaOAc: 261, 335. +NaOAc/$H_3BO_3$: 285, 343. The spectral data for the synthetic sample (**1**) were identical with those for the natural one (D-1).

**Synthesis of 6-(3-Phenylpropionyl)-5,7-dihydroxyneoflavanone (2)** According to the procedure described for the synthesis of **1**, 5,7-dibenzyloxy-6-cinnamoylneoflavone (1.2 g) was obtained by condensation of **6** (1.3 g, 2.7 mmol) with benzaldehyde (290 mg, 2.7 mmol), following by lactonization, as orange-yellow needles, mp 206—207 °C (MeOH). EIMS *m/z* (rel. int.): 564 ($M^+$, 16), 538 (3), 473 (100), 445 (36), 384 (24), 371 (11), 343 (70), 193 (32), 180 (8), 121 (67), 91 (100). $^1$H-NMR ($CDCl_3$, 270 MHz) $\delta$: 5.06, 5.07 (2H, each s, $OC\underline{H}_2Ph$), 6.20 (1H, s, H-3), 6.56 (1H, s, H-8), 7.03—7.50 (20H, m, 2 × $OCH_2\underline{Ph}$, 2 × Ph), 7.68, 7.85 (1H, each d, $J$=15.8 Hz, COCH=CH), 14.48 (1H, s, OH). The resulting neoflavone (400 mg) was hydrogenated to give **2** and 6-(3-phenylpropionyl)-5,7-dihydroxyneoflavone (**2′**). **2**: Colorless needles (180 mg), mp 170—171 °C (EtOAc–$C_6H_{14}$). EIMS *m/z* (rel. int.): 388 ($M^+$, 64), 370 (11), 360 (8), 345 (3), 327 (7), 283 (100), 256 (51), 241 (59), 228 (4), 213 (8), 179 (9), 155 (51), 121 (13), 115 (8), 104 (12), 91 (48). $^1$H-NMR (DMSO-$d_6$, 270 MHz) $\delta$: 2.87 (1H, dd, $J$=16.1, 1.8 Hz, H-3 *cis*), 2.91 (2H, t, $J$=7.7 Hz, $COCH_2$), 4.53 (1H, br d, $J$=7.0 Hz, H-4), 6.23 (1H, s, H-8), 7.07—7.31 (10H, m, 2 × Ph), 11.35, 13.91 (1H, each br s, OH). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 230sh (4.27), 280 (4.25), 334 (3.51). +NaOMe: 252sh, 329. +$AlCl_3$: 231sh, 281, 309sh, 357. +$AlCl_3$/HCl: 230sh, 281, 357. +NaOAc: 287, 374. +NaOAc/$H_3BO_3$: 282, 335. **2′**: Pale yellow needles (60 mg), mp 219—221 °C ($C_6H_6$). EIMS *m/z* (rel. int.): 386 ($M^+$, 36), 368 (9), 281 (100), 254 (22), 226 (6), 171 (8), 141 (5), 115 (9), 105 (6), 91 (24). $^1$H-NMR (DMSO-$d_6$, 270 MHz) $\delta$: 2.88 (2H, t, $J$=7.7 Hz, $COCH_2C\underline{H}_2$), 3.39 (2H, t, $J$=7.7 Hz, $COCH_2$), 5.38 (1H, s, H-3), 6.40 (1H, s, H-8), 7.13—7.41 (10H, m, 2 × Ph), 12.04, 14.75 (1H, each br s, OH). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 239sh (4.18), 282 (4.42), 339 (4.05), 406 (3.64). +NaOMe: 235sh, 295, 361, 406. +$AlCl_3$: 235sh, 282, 335sh. +$AlCl_3$/HCl: 235sh, 280, 335. +NaOAc: 255sh, 287, 370sh, 404. +NaOAc/$H_3BO_3$: 302, 376, 416.

**Synthesis of 8-(2-Carboxy-1-phenylethyl)-5,7-dihydroxyflavone $\delta$-Lactone (3)** A 2-methoxyethanol solution (50 ml) containing 3′-(2-carboxy-1-phenylethyl)-2′-hydroxy-4′,6′-diisopropoxychalcone (2.5 g, 5 mmol) and $H_3PO_4$ (5 ml) was refluxed for 4 h to give the corresponding methoxyethyl ester (2.4 g) as pale yellow crystals. EIMS *m/z* (rel. int.): 529 ($M^+$ − 15, 100), 502 (8), 385 (9), 355 (18), 343 (71), 325 (4), 281 (8), 241 (18), 131 (18). A dry dioxane solution (25 ml) containing the above ester (1.7 g, 3 mmol) and DDQ (1.7 g, 7 mmol) was refluxed for 17 h. After cooling, the precipitate of reduced DDQ was filtered off and the filtrate was subjected to silica gel column chromatography (eluent: $C_6H_6$–EtOAc, 5 : 1) to give **9** (325 mg) and **10** (280 mg). **9**: An amorphous powder. EIMS *m/z* (rel. int.): 544 ($M^+$, trace), 529 ($M^+$ − 15, 100), 502 (8), 385 (40), 327 (16), 343 (100), 325 (16), 241 (24), 131 (26). **10**: An amorphous powder. EIMS *m/z* (rel. int.): 542 ($M^+$, 73), 527 ($M^+$ − 15, 100), 500 (50), 458 (37), 383 (79), 354 (66), 307 (68), 252 (42), 131 (47). $^1$H-NMR (DMSO-$d_6$,

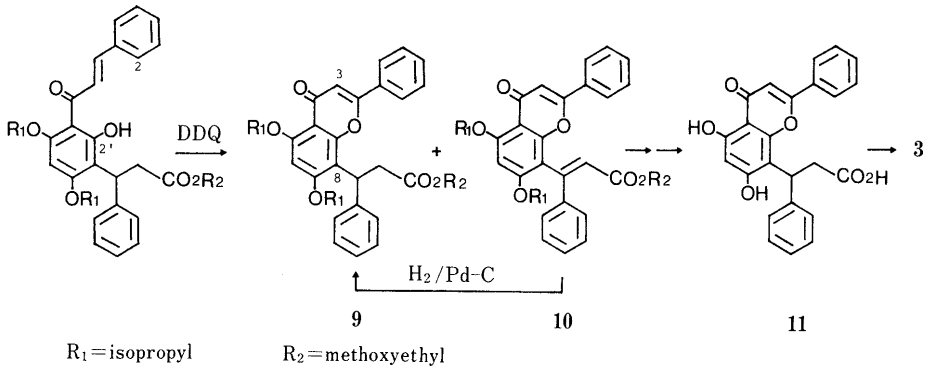



Chart 3

270 MHz) $\delta$: 0.93, 1.13 (3H, each d, $J$=5.9 Hz, $CH_3$), 1.40 (6H, d, $J$=5.9 Hz, 2×$CH_3$), 3.09 (3H, s, $OCH_3$), 3.30 (2H, m, $CO_2CH_2$), 3.98 (2H, m, $CH_2OCH_3$), 4.80 (2H, m, 2×OCH<), 6.66, 6.67, 6.70 (1H, each s, H-3, 6 and C=CH), 7.35—7.71 (10H, m, side phenyl). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1705, 1650, 1620, 1590. UV $\lambda_{max}^{MeOH}$ nm: 270, 325sh. An EtOAc solution containing **10** (325 mg, 0.6 mmol) and 10% Pd–C (33 mg) was stirred under an $H_2$ atmosphere for 2 h to give **9** (300 mg). A 70% EtOH solution containing **9** (500 mg, 0.9 mmol) and KOH (2 g) was refluxed for 2 h. The reaction mixture was acidified with 5% HCl to precipitate colorless crystals (210 mg), mp 214—215 °C. EIMS $m/z$ (rel. int.): 486 ($M^+$, trace), 471 (99), 444 (70), 429 (9), 385 (29), 368 (22), 343 (100), 327 (16). $^1$H-NMR ($CDCl_3$, 270 MHz) $\delta$: 1.31, 1.40 (6H, each d, $J$=6.0 Hz, $CH_3$), 3.25 (1H, dd, $J$=15.8, 8.4 Hz, CHCOOH), 3.40 (1H, dd, $J$=15.8, 6.9 Hz, CHCOOH), 4.57 (2H, m, CH<), 5.51 (1H, dd, $J$=8.4, 6.9 Hz, Ph-CH), 6.35, 6.60 (1H, s, H-3 and 6), 7.12—7.85 (10H, m, side phenyl). A $CH_2Cl_2$ solution containing the crystals (200 mg, 0.4 mmol) obtained above was cooled at −60 °C, and $BCl_3$ (0.5 ml) was added to the solution. The reaction mixture was left at room temperature for 2 h, and poured into water. The solution was extracted with EtOAc to give **11** as a colorless amorphous powder. EIMS $m/z$ (rel. int.): 384 ($M^+$, 100), 356 (26), 341 (39), 307 (53), 238 (11), 205 (29). A benzene solution containing **11** (100 mg, 0.24 mmol), $Ac_2O$ (2 ml) and fused sodium acetate (1 g) was refluxed for 2 h. The reaction mixture was poured into water and extracted with EtOAc. The EtOAc extract was purified by column chromatography on silica gel (eluent: $C_6H_6$–acetone, 5:1) to give **3** (78 mg) as pale yellow rectangles, mp 294 °C ($C_6H_6$–EtOAc). EIMS $m/z$ (rel. int.): 384 ($M^+$ for $C_{24}H_{16}O_5$, 100), 356 (20), 341 (36), 307 (47), 205 (27). $^1$H-NMR (acetone-$d_6$, 270 MHz) $\delta$: 3.15 (2H, m, $CHCH_2CO$), 4.85 (1H, dd, $J$=6.2, 2.9 Hz, Ph-CH), 6.66, 6.70 (1H, each s, H-3 and 6), 7.21—7.67 (10H, m, 2×side phenyl), 12.75 (1H, s, $C_5$-OH). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1780 (lactone C=O), 1650 (C=O), 1610, 1590. UV $\lambda_{max}^{MeOH}$ nm: 273, 313sh, 335. +NaOMe: 245, 265sh, 282, 380. +$AlCl_3$: 273, 292, 325, 380. +$AlCl_3$/HCl: 281sh, 291, 322, 376. +AcONa: 273, 295inf, 313sh. +AcONa/$H_3BO_3$: 273, 297sh, 315sh. The spectral data for the synthetic sample were identical with those for the natural one (D-2a).

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**References and Notes**

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8) The signal of H-6 of neoflavone appears at higher field than that of H-8 [8-acetyl-5,7-dihydroxyneoflavone $^1$H-NMR (DMSO-$d_6$, 60 MHz) $\delta$: 5.90 (1H, s, H-3), 6.14 (1H, s, H-6); 6-acetyl-5,7-dihydroxyneoflavone $^1$H-NMR (DMSO-$d_6$, 60 MHz) $\delta$: 5.84 (1H, s, H-3), 6.40 (1H, s, H-8)]: see ref. 5.
9) A natural sample (D-2a) exhibited two spots on TLC (eluent: hexane–acetone, 2:1), one of which was completely superimposed on that of the synthetic sample of **3** (*Rf* 0.55). The other spot, which was yellow (*Rf* 0.53), may be that of a flavonol-type compound as described in ref. 1.