[Chem. Pharm. Bull.
33(3) 971—974 (1985)]

Formation of Pigments by the Tissue Culture of *Cassia occidentalis*[1)]

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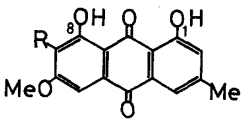
(Received May 28, 1984)

From the callus of *Cassia occidentalis* LINN., six anthraquinones [islandicin, chrysophanol, physcion, emodin, questin, and 7-methylphyscion (**1**)], a bianthrone (chrysophanol-10,10′-bianthrone), three tetrahydroanthracenes [germichrysone, methylgermitorosone, and 7-methyltorosachrysone (**2**)], and a xanthone (pinselin) were isolated. The structures of the two new compounds, **1** and **2**, were established on the basis of spectral and chemical evidence.

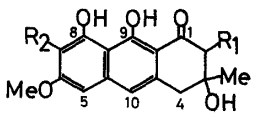
Keywords——*Cassia occidentalis*; Leguminosae; tissue culture; callus; 7-methylphyscion; 7-methyltorosachrysone

The seed of *Cassia occidentalis* LINN. ("wangjiangnanzi" in Chinese) is used in Chinese medicine as a mild purgative and a stomachic. A number of workers have chemically investigated the seeds,[2–11)] roots,[12–15)] leaves,[16–18)] and flowers[19)] of this plant. In the present paper, we wish to report on the structures of the pigments isolated from the callus of *C. occidentalis*.

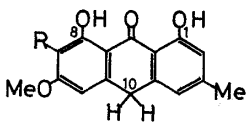
The callus culture was derived from seedlings, and it was maintained on a Murashige–Skoog's medium (MS medium) containing 2,4-D and kinetin. The benzene extract of the fresh callus was treated as described in Experimental, and two new pigments, 7-methylphyscion (**1**) and 7-methyltorosachrysone (**2**) were isolated, along with islandicin,[20)] chrysophanol, physcion, emodin, germichrysone,[21)] questin, chrysophanol-10,10′-bianthrone,[22)] and methylgermitorosone.[23)]



1: R=Me
3: R=H



2: R_1=H, R_2=Me
4: R_1=H, R_2=H
5: R_1=OH, R_2=Me



6: R=Me
7: R=H

Chart 1

7-Methylphyscion (**1**), obtained in the form of yellow needles, mp 233—233.5 °C, $C_{17}H_{14}O_5$ (*m/z* 298, M^+), was considered to be an anthraquinone derivative judging from the results of color reactions with aqueous sodium hydroxide solution and methanolic magnesium acetate.[24)] The ultraviolet (UV) maximum at 434 nm of **1** showed it to have a 1,8-dihydroxy structure.[25)] The two bands at 1670 and 1620 cm^{-1} in its infrared (IR) spectrum suggested the presence of a nonchelated carbonyl group and a chelated carbonyl group respectively. The

TABLE I. ^{1}H-NMR Data for Compounds **1**—**7**[a]

	1	**3**	**6**	**7**	**2**	**4**	**5**
C_2-H	7.07 br s	7.04 br s	6.68 br s	6.67 br s	2.84 br s	2.83 br s	4.28 d (J=1.5 Hz)
C_3-Me	2.45 br s	2.45 br s	2.36 br s	2.34 br s	1.45 s	1.45 s	1.53 s
C_4-H	7.62 br s	7.57 br s	6.68 br s	6.67 br s	3.04 br s	3.03 br s	2.12 br s
C_{10}-H			4.26 br s		6.87 s	6.86 s	6.89 s
C_5-H	7.40 s	7.32 d (J=2.4 Hz)	6.42 br s	6.39 s	6.53 s	6.54 d (J=2.4 Hz)	6.53 s
C_6-OMe	4.02 s	3.92 s	3.91 s	3.85 s	3.92 s	3.88 s	3.90 s
C_7-H		6.60 d (J=2.4 Hz)		6.39 s		6.47 d (J=2.4 Hz)	
C_7-Me	2.19 s		2.10 s		2.18 s		2.17 s
C_8-OH	12.45 s	12.26 s	12.70 s	12.62 s	9.97 s	9.79 s	9.58 s
C_1-OH	12.15 s	12.05 s	12.41 s	12.31 s			
C_2-OH							3.84 d (J=1.5 Hz)
C_9-OH					16.13 s	16.10 s	14.61 s

a) Measured in $CDCl_3$ at 100 MHz, with TMS as the internal standard. The following abbreviations are used: s, singlet; br s, broad singlet; d, doublet.

proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** showed the presence of two methyl groups, an aromatic methoxyl group, three aromatic protons, and two chelated hydroxyl groups (Table I). The assignments of the two methyl groups were performed on the basis of spin-decoupling experiments. The irradiation of the methyl signal at δ 2.45 ppm changed the two broad singlets at δ 7.07 and 7.62 ppm to two sharp doublets (J=2.4 Hz), whereas irradiation of the other methyl singlet at δ 2.19 caused no change. The above data indicate that **1** has one more aromatic methyl group than does physicon (**3**). From these spectral data, the structure of **1** was established to be 1,8-dihydroxy-6-methoxy-3,7-dimethylanthraquinone (7-methylphyscion).

7-Methyltorosachrysone (**2**) was obtained as yellow prisms; mp 208—210 °C. The high-resolution mass spectrum (MS) gave $C_{17}H_{18}O_5$ as the molecular formula, and major fragment ions were obtained at m/z 284 ($M^+ - H_2O$), 268 ($M^+ - H_2O - Me$), 260 ($M^+ - COCH_2$), 244 ($M^+ - CH_2CMeOH$), and 241 ($M^+ - H_2O - Me - CO$). The similarity of the chromophore of **2** to those of torosachrysone (**4**)[26] and germitorosone (**5**)[23] was suggested by the UV and IR spectra; thus, **2** was assumed to be a tetrahydroanthracene derivative. The band at 1630 cm^{-1} revealed the presence of a chelated carbonyl group, while the UV maxima at 303, 316, and 328 nm showed that **2** possesses a naphthalene skeleton. The ^{1}H-NMR spectrum of **2** showed the presence of two methylene groups, two methyl groups (aromatic and aliphatic), an aromatic methoxyl group, two aromatic protons, and three hydroxyl groups, including two chelated hydroxyl groups (Table I). The above data indicate that **2** has one more aromatic methyl group and one less aromatic proton than **4**, and one more methylene group and one less aliphatic methine proton than **5**.

Compound **2** was converted to **6** (mp 240—242 °C) on treatment with hydrogen chloride–acetic acid. The UV maxima of **6** at 238, 254 sh, 263 sh, 272, 320, and 351 nm suggested that **6** has an anthrone skeleton, while the ^{1}H-NMR spectrum of **6** showed the presence of two methyl groups, a methoxyl group, a methylene group, three aromatic protons, and two hydroxyl groups. The MS of **6** gave $C_{17}H_{16}O_4$ as the molecular formula. In spin-decoupling experiments, two aromatic protons, δ 6.68 (C_4-H) and 6.42 (C_5-H), showed long-range spin couplings with methylene protons at δ 4.26 (C_{10}-H). The ^{1}H-NMR data showed that **6** had

one more methyl group than physcion-9-anthrone (**7**). The structure of **6** was established as 1,8-dihydroxy-6-methoxy-3,7-dimethyl-9-anthrone (7-methylphyscion-9-anthrone). The conversion from **6** to **1** by treatment with 5% sodium hydroxide–methanol established the structure of **2** as 7-methyltorosachrysone (3,8,9-trihydroxy-6-methoxy-3,7-dimethyl-1-oxo-1,2,3,4-tetrahydroanthracene).

It is interesting from a biogenetic point of veiw that **1** and **2**, each with two β-methyl substituents on the anthracene skeleton, coexist in the callus of *C. occidentalis*. The anthraquinone and tetrahydroanthracene derivatives in this plant may be formed from the same anthrones. Thus, the oxidation of **6** would lead to **1**, and the addition of water to **6** could give **2**. These compounds may be formed by the acetate-malonate pathway.

Experimental

All the melting points were taken on a Yanagimoto micro-melting-point apparatus and are uncorrected. The UV spectra were obtained on a Hitachi 200-10 spectrophotometer, and the IR spectra were recorded on a JASCO IR A-2 spectrophotometer. The NMR spectra were taken on a JEOL FX-100 instrument; the chemical shifts are given in ppm relative to internal tetramethylsilane (TMS). The MS were obtained on a Hitachi RMU-7M spectrometer. Column chromatography was performed on silicic acid (SiO_2) (Mallinckrodt).

Origin of Callus Tissue——The seeds of *Cassia occidentalis* LINN., collected at the Drug Plant Garden of the College of Science and Technology, Nihon University, were washed with a neutral detergent solution, rinsed with water, soaked for 5 min in 70% aqueous EtOH, and rinsed again with sterile distilled water. The seeds were then aseptically transferred to sterile 100 ml Erlenmeyer flasks containing 30 ml of a sucrose agar culture medium (30 g of sucrose and 10 g of agar in 1 l of distilled water). The hypocotyl segments or stems excised from 5-d-old seedlings, were planted aseptically on 40 ml of MS medium containing 3% sucrose, 1% agar, 2,4-D (1 ppm), and kinetin (0.1 ppm) in 100 ml Erlenmeyer flasks. Two to four weeks later, the dark brown callus tissues thus induced were removed from the original plant tissues and transferred onto the MS medium. The callus culture was grown in the dark at 25 °C and subcultured regularly at 4-week intervals.

Extraction and Isolation of the Pigments of Callus Tissue——The callus tissue (421 g; fresh weight) was extracted with C_6H_6 three times for 3 h each time, and then the extract was concentrated *in vacuo* to give a yellow-brown mass (1.1 g). The mass was chromatographed on SiO_2 with C_6H_6–AcOEt (1 : 0→4 : 1) as the solvent, to give fractions 1—4. Fraction 1 was rechromatographed on SiO_2 with *n*-hexane–AcOEt (49 : 1) as the solvent system to afford islandicin (1 mg), chrysophanol (24 mg), 7-methylphyscion (**1**) (1 mg), and physcion (2 mg). Fraction 2 was rechromatographed with C_6H_6–AcOEt (19 : 1) as the solvent system to afford chrysophanol-10,10′-bianthrone (78 mg), emodin (8 mg), pinselin (136 mg), and questin (3 mg). Fraction 3 was rechromatographed with $CHCl_3$–AcOEt (4 : 1) as the solvent system to afford 7-methyltorosachrysone (**2**) (22 mg), germichrysone (85 mg), and methylgermitorosone (1 mg). The known compounds were identified by direct comparison of the IR spectra with those of authentic samples.

7-Methylphyscion (1)——Compound **1** was recrystallized from $CHCl_3$ to yield yellow needles; mp 233—233.5 °C. UV $\lambda_{max}^{dioxane}$ nm (log ε): 238 sh (4.15), 246 sh (4.21), 273 (4.52), 307 (4.04), 434 (4.12), 455 sh (4.09). IR ν_{max}^{KBr} cm^{-1}: 1670, 1620, 1610, 1570, 1555, 1480. MS *m/z*: 298 (M^+, 100%), 283 ($M^+ - Me$, 19), 280 ($M^+ - H_2O$, 58), 269 ($M^+ - CHO$, 27). High-resolution MS *m/z*: Calcd for $C_{17}H_{14}O_5$: 298.0840. Found: 298.0827. The ^{1}H-NMR data are given in Table I.

7-Methyltorosachrysone (2)——Compound **2** was recrystallized from AcOEt to yield yellow prisms; mp 208—210 °C. UV $\lambda_{max}^{dioxane}$ nm (log ε): 227 (4.47), 270 sh (4.61), 277 (4.47), 303 (3.76), 316 (3.91), 328 (3.83), 397 (4.00). IR ν_{max}^{KBr} cm^{-1}: 3300, 2950, 1630, 1580, 1510. MS *m/z*: 302 (M^+, 100%), 284 ($M^+ - H_2O$, 11), 269 ($M^+ - H_2O - Me$, 15), 260 ($M^+ - COCH_2$, 15), 244 ($M^+ - CH_2CMeOH$, 17), 241 ($M^+ - H_2O - Me - CO$, 12). High-resolution MS *m/z*: Calcd for $C_{17}H_{18}O_5$: 302.1153. Found: 302.1138. The ^{1}H-NMR data are given in Table I.

7-Methylphyscion-9-anthrone (6) from 7-Methyltorosachrysone (2)——Compound **2** (4 mg) was dissolved in a mixture of AcOH (1 ml) and conc. HCl (0.01 ml), and the solution was heated at 80 °C for 1 h, then diluted with H_2O and extracted with AcOEt. The extract was evaporated *in vacuo*, and the yellow residue was recrystallized from C_6H_6 to give yellow needles; mp 240—242 °C. UV $\lambda_{max}^{dioxane}$ nm (log ε): 238 (3.90), 252 sh (3.75), 263 sh (3.72), 272 (3.84), 320 (3.92), 351 (4.09). IR ν_{max}^{KBr} cm^{-1}: 3450, 2930, 1620, 1595, 1480. MS *m/z*: 282 (M^+, 100%), 269 ($M^+ - Me$, 42), 255 ($M^+ - CO$, 8), 254 ($M^+ - CHO$, 7), 253 ($M^+ - CH_2O$, 9), 241 ($M^+ - OMe$, 19). High-resolution MS *m/z*: Calcd for $C_{17}H_{16}O_4$: 284.1047. Found: 284.1037. The ^{1}H-NMR data are given in Table I.

7-Methylphysicon (1) from 7-Methylphyscion-9-anthrone (6)——Compound **6** (2 mg) was dissolved in a 5% aqueous NaOH solution (5 ml), and the solution was left to stand at room temperature for 3 h, then neutralized with 1% HCl, diluted with H_2O and extracted with AcOEt. The extract was concentrated *in vacuo*, and the yellow residue was recrystallized from C_6H_6 to give yellow needles (1 mg); mp 241—242 °C. The crystals were identified as **1** by

means of mmp determination and IR comparison.

Acknowledgements We wish to thank Miss Y. Kimura of the Department of Pharmacy, Nihon University, for the IR spectra, and Mr. M. Aimi and Dr. T. Takido of the Analytical Center, College of Science and Technology, Nihon University, for the MS and NMR spectra.

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