[Chem. Pharm. Bull. 32(3) 860—864 (1984)]

# Studies on the Constituents of the Seeds of *Cassia obtusifolia* LINN. The Structures of Three New Anthraquinones[1)]

SUSUMU KITANAKA* and MICHIO TAKIDO

*Department of Pharmacy, College of Science and Technology, Nihon University, 1–8, Kanda-surugadai, Chiyoda-ku, Tokyo 101, Japan*

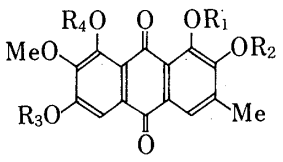
(Received June 14, 1983)

Three new anthraquinones, 1-desmethylchryso-obtusin (**4**), 1-desmethylobtusin (**5**) and 1-desmethylaurantio-obtusin (**6**), were isolated along with chrysophanol-10,10′-bianthrone, questin and benzoic acid from the seeds of *Cassia obtusifolia* LINN., and their structures were established on the basis of spectral and chemical evidence.

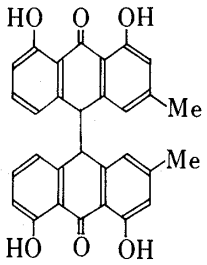
**Keywords**——*Cassia obtusifolia*; Leguminosae; anthraquinone; $^1$H-NMR; acylation shift

Cassia seed (*Cassia obtusifolia* LINN., Leguminosae) is called Ketsumeishi in Japan, and is used as a mild purgative, tonic and diuretic. In previous papers, we reported the isolation of chrysophanol, physcion, obtusifolin,[2)] chryso-obtusin (**1**), obtusin (**2**), aurantio-obtusin (**3**),[3)] gluco-obtusifolin, gluco-aurantio-obtusin,[4)] rubrofusarin, cassiaside,[5)] torosachrysone, isotoralactone and cassialactone[6)] from the seeds of *C. obtusifolia*. In this paper, we report the structural determination of three new anthraquinones, 1-desmethylchryso-obtusin (**4**), 1-desmethylobtusin (**5**) and 1-desmethylaurantio-obtusin (**6**), isolated along with chrysophanol-10,10′-bianthrone (**7**), questin (**8**) and benzoic acid from the seeds of this plant.

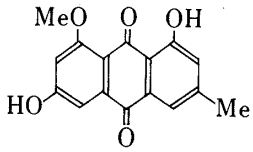
The benzene and methanol extracts of the crushed seeds were treated as described in Experimental.



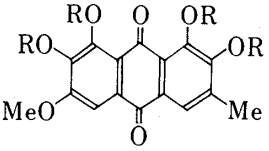
**1** : $R_1$, $R_3$, $R_4$=Me, $R_2$=H
**2** : $R_1$, $R_3$=Me, $R_2$, $R_4$=H
**3** : $R_1$=Me, $R_2$, $R_3$, $R_4$=H
**4** : $R_3$, $R_4$=Me, $R_1$, $R_2$=H
**5** : $R_3$=Me, $R_1$, $R_2$, $R_4$=H
**6** : $R_1$, $R_2$, $R_3$, $R_4$=H
**9** : $R_1$, $R_2$, $R_3$, $R_4$=Me
**1a** : $R_1$, $R_3$, $R_4$=Me, $R_2$=Ac
**2a** : $R_1$, $R_3$=Me, $R_2$, $R_4$=Ac
**3a** : $R_1$=Me, $R_2$, $R_3$, $R_4$=Ac
**4a** : $R_3$, $R_4$=Me, $R_1$, $R_2$=Ac
**5a** : $R_3$=Me, $R_1$, $R_2$, $R_4$=Ac
**6a** : $R_1$, $R_2$, $R_3$, $R_4$=Ac



**7**



**8**



**10** : R=H
**10a** : R=Ac

Chart 1

Compound **7**, yellow needles, mp 221—222 °C, $C_{32}H_{26}O_8$ (*m/z* 478, $M^+$), was identified as chrysophanol-10,10′-bianthrone by direct comparison with a synthetically prepared sample.[7]

Questin (**8**),[8] yellow needles, mp 300—303 °C, $C_{16}H_{12}O_6$ (*m/z* 284, $M^+$) and benzoic acid, colorless plates, mp 121—124 °C, $C_7H_6O_2$ (*m/z* 122, $M^+$) were identified by direct comparisons with authentic samples.

Compounds **4**—**6** were identified as three anthraquinone derivatives having vicinal hydroxy groups. All of them showed a purple color in methanolic magnesium acetate,[9] and all of them gave the same compound, 1,2,6,7,8-pentamethoxy-3-methylanthraquinone (**9**)[3] upon methylation. The structures of the above compounds were investigated by comparison of acylation shifts[10] in the proton nuclear magnetic resonance ($^1$H-NMR) spectra of known 1,2,6,7,8-penta-*O*-substituted 3-methylanthraquinones (1—3, and 10[3]) and their peracetates (Table I). Significant differences were found for the downfield shifts of aromatic nuclear protons on *para* (0.24—0.43 ppm), or both *ortho* and *para* positions (about 0.5 ppm) with respect to *O*-acetyl groups, while the differences were small in *meta* positions (0 to 0.04 ppm),

Table I. $^1$H-NMR Data for Hydroxyanthraquinones (**1**—**6**, **9**, **10**) and Their Peracetates (**1a**—**6a**, **10a**)[a,b]

| | **1**, (**1a**) | **2**, (**2a**) | **3**, (**3a**) | **4**, (**4a**) | **5**, (**5a**) | **6**, (**6a**) | **9** | **10** (**10a**) |
|---|---|---|---|---|---|---|---|---|
| H-4 | 7.88 br s | 7.95 br s | 7.94 br s | 7.63 br s | 7.68 br s | 7.68 br s | 7.86 q | 7.67 br s |
| | (7.92) br s | (7.95) br s | (7.95) br s | (8.06) br s | (8.03) br s | (8.09) br s | *J*=0.7 | (8.09) br s |
| H-5 | 7.61 s | 7.42 s | 7.39 s | 7.70 s | 7.48 s | 7.45 s | 7.58 s | 7.50 s |
| | (7.58) s | (7.70) s | (7.92) s | (7.61) s | (7.72) s | (7.94) s | | (7.76) s |
| Me-3 | 2.38 br s | 2.40 br s | 2.39 br s | 2.37 br s | 2.38 br s | 2.38 br s | 2.37 d | 2.38 br s |
| | (2.32) br s | (2.32) br s | (2.32) br s | (2.34) br s | (2.35) br s | (2.35) br s | *J*=0.7 | (2.37) br s |
| OH | 6.79 s | 6.78 s | 6.37 s | 6.37 s | 6.36 s | 6.31 s | | 5.89 s |
| | | 13.03 s | 6.67 s | 13.26 s | 12.13 s | 6.57 s | | 6.22 s |
| | | | 13.30 s | | 12.21 s | 12.20 s | | 11.94 s |
| | | | | | | 12.42 s | | 12.11 s |
| OAc | (2.41) s | (2.40) s | (2.39) s | (2.39) s | (2.38) s | (2.38) s | | (2.35) s×2 |
| | | (2.50) s | (2.40) s | (2.48) s | (2.43) s | (2.39) s | | (2.42) s×2 |
| | | | (2.51) s | | (2.46) s | (2.43) s | | |
| | | | | | | (2.46) s | | |
| OMe | 3.99 s | 4.02 s×2 | 4.02 s | 3.99 s | 4.01 s | 4.12 s | 3.98 s×2 | 4.07 s |
| | 4.01 s | 4.04 s | 4.12 s | 4.01 s | 4.05 s | (3.94) s | 3.99 s | (4.02) s |
| | 4.02 s | (3.88) s | (3.87) s | 4.05 s | (3.93) s | | 4.01 s | |
| | 4.03 s | (3.95) s | (3.95) s | (3.93) s | (4.06) s | | 4.02 s | |
| | (3.95) s | (4.05) s | | (3.99) s | | | | |
| | (3.99) s×2 | | | (4.03) s | | | | |
| | (4.03) s | | | | | | | |

*a*) Measured in $CDCl_3$ at 100 MHz with TMS as an internal standard. s, singlet; br s, broad singlet; q, quartet.
*b*) The numerals in parentheses indicate chemical shifts in the acetates.

Table II. Acylation Shifts ($\Delta_{Ac}$*) of Hydroxyanthraquinones **1**—**6** and **10**

| Proton No. | 1 | 2 | 3 | 4 | 5 | 6 | 10 |
|---|---|---|---|---|---|---|---|
| H-4 | −0.04 | 0 | −0.01 | −0.43 | −0.35 | −0.41 | −0.42 |
| H-5 | +0.03 | −0.28 | −0.53 | +0.09 | −0.24 | −0.49 | −0.26 |

$\Delta_{Ac}{}^* = \delta_{\text{hydroxyanthraquinone}} - \delta_{\text{peracetylanthraquinone}}$

and the acylation shifts ($\Delta_{Ac}$*) (Table II) provide a good indication of the positions of *O*-acetyl groups.

Compound **4**, 1-desmethylchryso-obtusin, orange-red needles, mp 211—212.5 °C, $C_{18}H_{16}O_7$ (*m/z* 344, $M^+$) showed a chelated phenolic hydroxy at $\delta$ 13.26, a phenolic hydroxyl at $\delta$ 6.37 and three methoxyl signals in the $^1$H-NMR spectrum, so that its structure is either 1,2-dihydroxy-6,7,8-trimethoxyl-3-methylanthraquinone or 7,8-dihydroxy-1,2,6-trimethoxy-3-methylanthraquinone. The H-4 proton signal coupled with methyl protons of the peracetate (**4a**) was shifted downfield by 0.43 ppm relative to that of **4**. Therefore, the structure of compound **4** was established to be 1,2-dihydroxy-6,7,8-trimethoxy-3-methylanthraquinone.

Compound **5**, 1-desmethylobtusin was isolated as orange needles, mp 260—263 °C, $C_{17}H_{14}O_7$ (*m/z* 330, $M^+$). The $^1$H-NMR spectrum of **5** indicated the presence of two chelated phenolic hydroxyls ($\delta$ 12.13 and 12.21), a phenolic hydroxyl ($\delta$ 6.36) and two methoxy groups. The signals of H-4 and H-5 in the triacetate (**5a**) showed downfield shifts of 0.35 and 0.24 ppm, respectively, relative to **5**. From these results and biogenetic considerations we assumed that the structure is 6,7-dimethoxy-1,2,8-trihydroxy-3-methylanthraquinone. An authentic sample was synthesized by partial demethylation of obtusin with sulfuric acid and shown to be identical with **5**. Therefore, the structure of compound **5** was determined as 6,7-dimethoxy-1,2,8-trihydroxy-3-methylanthraquinone.

Compound **6**, 1-desmethylaurantio-obtusin, yellow-brown prisms, mp >300 °C, $C_{16}H_{12}O_7$ (*m/z* 316, $M^+$) showed signals of two chelated phenolic hydroxyls at $\delta$ 6.31 and 6.57, and a methoxyl in the $^1$H-NMR spectrum. The signals of H-4 and H-5 in the tetraacetate (**6a**) were shifted downfield by 0.31 and 0.49 ppm, respectively, relative to **6**. From these results the structure of **6** was considered to be 1-desmethylaurantio-obtusin. An authentic sample was synthesized by partial demethylation of **3** with hydrobromic acid. Compound **6** and the synthetic sample were identical. Thus, compound **6** is 7-methoxy-1,2,6,8-tetrahydroxy-3-methylanthraquinone.

It is interesting from a biogenetic point of view that these three new anthraquinones are the 1-desmethyl compounds of three anthraquinones which were isolated previously from these seeds.

## Experimental

All melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. The ultraviolet (UV) spectra were obtained on a Hitachi 200—10 spectrophotometer and the infrared (IR) spectra were recorded on a JASCO IR A-2 spectrophotometer. The NMR spectra were taken on a JEOL FX-100 instrument with $CDCl_3$ as the solvent, and the chemical shifts are given in ppm relative to internal tetramethylsilane (TMS). Mass spectra (MS) were obtained on a Hitachi RMU-7M spectrometer. Column chromatography was performed on silicic acid (Mallinckrodt), and polyamide (Wako C-200, Wako Pure Chemical Ind., Ltd.).

**Extraction and Isolation**——Plant material was obtained from the Drug Plant Garden of the College of Science and Technology, Nihon University, and a specimen having ripe seeds has been preserved in the herbarium of our laboratory.

Crushed *Cassia obtusifolia* seeds (1 kg) were added with $H_2O$ (1 l), and were extracted with benzene ($C_6H_6$) (3 × 8 l). The $C_6H_6$ extract was concentrated *in vacuo* and the residue was chromatographed on silicic acid with $C_6H_6$ to afford a mixture of chrysophanol-10,10′-bianthrone (**7**) and isotoralactone. Compound **7** (90 mg) was isolated from the mixture by rechromatography using *n*-hexane–ethylacetate (AcOEt) (9 : 1), and was identified as chrysophanol-10,10′-bianthrone by comparison with a synthetically prepared sample. Crushed seeds (5 kg) were extracted with 70% MeOH (2 × 35 l) and the filtrate was concentrated *in vacuo* to a syrup. This was partitioned several times with $C_6H_6$ and then AcOEt. The $C_6H_6$ extract was concentrated *in vacuo* and chromatographed on silicic acid with $C_6H_6$–AcOEt (19 : 1) to afford chrysophanol, physcion, isotoralactone, rubrofusarin, obtusifolin, obtusin and a mixture of **4** and **5**, and then with $C_6H_6$–AcOEt (4 : 1) to afford chryso-obtusin, a mixture of aurantio-obtusin and **6**, and a mixture of questin (**8**) and benzoic acid, successively. The mixture of **4** and **5** was separated to yield **4** (16 mg) and **5** (4 mg) by column chromatography on polyamide with 80% MeOH, respectively. The mixture of aurantio-obtusin and **6** was chromatographed on polyamide with 70% MeOH and **6** (300 mg) was obtained in the latter eluate. From the mixture

of **8** and benzoic acid, benzoic acid (1 mg) was deposited as leaflets upon cooling, and the remainder was recrystallized from MeOH to afford **8** (20 mg) which was identified as questin by direct comparison with an authentic sample.

**1-Desmethylchryso-obtusin (4)**——Compound **4** was recrystallized from MeOH to yield orange-red needles, mp 211—212.5 °C. UV $\lambda_{max}^{dioxane}$ nm (log ε): 278 (4.76), 316 (4.12), 400 (3.86). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3300, 1660, 1630, 1580. MS *m/z*: 344 ($M^+$, 100%), 327 ($M^+ - CH_3$, 8), 326 ($M^+ - H_2O$, 78), 273 ($M^+ - CH_3 - H_2O - CO$, 11), 255 ($M^+ - CH_3 - H_2O - 2\,CO$, 10). High resolution MS *m/z*: Calcd for $C_{18}H_{16}O_7$: 344.0894. Found: 344.0869. $^1$H-NMR data are shown in Table I.

**Acetate (4a) of 4**——**4** (2 mg) gave a diacetate (**4a**) upon acetylation with $Ac_2O$–pyridine; the product was recrystallized from MeOH to give yellow needles (1.5 mg), mp 204—205 °C. UV $\lambda_{max}^{dioxane}$ nm (log ε): 243 (sh, 4.20), 249 (4.22), 278 (4.62), 343 (3.74). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 1780, 1670, 1605, 1580, 1570. High resolution MS *m/z*: Calcd for $C_{22}H_{20}O_9$: 428.1106. Found: 428.1139. $^1$H-NMR data are shown in Table II.

**Methylation of 4**——$Me_2SO_4$ (0.1 ml) and $K_2CO_3$ (100 mg) were added to a solution of **4** (2 mg) in $Me_2CO$ (1 ml), and the whole was refluxed for 4 h, then cooled. The reaction mixture was treated with a few drops of $NH_4OH$ solution, then $H_2O$ was added, and the whole was extracted with ether. The extract was concentrated, and recrystallized from MeOH as pale yellow needles, mp 137—140 °C, which were identified by direct comparison with an authentic sample of 1,2,6,7,8-pentamethoxy-3-methylanthraquinone.

**1-Desmethylobtusin (5)**——Compound **5** was recrystallized from MeOH to yield orange needles, mp 260—263 °C. UV $\lambda_{max}^{dioxane}$ nm (log ε): 282 (4.56), 310 (sh, 3.87), 320 (3.90), 410 (3.96). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3400, 1670, 1620, 1580. High resolution MS *m/z*: Calcd for $C_{16}H_{12}O_7$: 316.0582. Found: 316.0598. $^1$H-NMR data are shown in Table I.

**Acetate (5a) of 5**——Compound **5** (2 mg) was acetylated with $Ac_2O$–pyridine to give a triacetate (**5a**), which was recrystallized from MeOH to give yellow needles (2 mg), mp 230—231 °C. UV $\lambda_{max}^{dioxane}$ nm (log ε): 243 (4.14), 250 (4.17), 276 (4.63), 340 (3.71). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 1775, 1760, 1675, 1660, 1585. High resolution MS *m/z*: Calcd for $C_{23}H_{20}O_{10}$: 456.1054. Found: 456.1028. $^1$H-NMR data are shown in Table II.

**Methylation of 5**——$Me_2SO_4$ (0.1 ml) and $K_2CO_3$ (100 mg) were added to a solution of **5** (1 mg) in $Me_2CO$ (1 ml), and the mixture was refluxed for 4 h, then treated as above. The permethyl ether of **5** was identified as 1,2,6,7,8-pentamethoxy-3-methylanthraquinone by direct comparison with an authentic sample.

**Conversion of Obtusin to 5**——Obtusin (10 mg) dissolved in 75% $H_2SO_4$ was heated at 100 °C for 15 min, then diluted with $H_2O$, and extracted with AcOEt. The extract was evaporated *in vacuo* and the residue was chromatographed on silicic acid with $C_6H_6$–AcOEt (9 : 1). The product was recrystallized from MeOH to afford orange needles (3.5 mg), mp 262—264 °C. The crystals were identified as **5** by mp, IR, $^1$H-NMR and MS.

**1-Desmethylaurantio-obtusin (6)**——The crude crystals were recrystallized from $Me_2CO$ to give yellow-brown prisms, mp >300 °C. UV $\lambda_{max}^{dioxane}$ nm (log ε): 282 (4.56), 310 (sh, 3.87), 320 (3.90), 410 (3.96). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3400, 1670, 1620, 1580. High resolution MS *m/z*: Calcd for $C_{16}H_{12}O_7$: 316.0582. Found: 316.0598. $^1$H-NMR data are shown in Table I.

**Acetate (6a) of 6**——Compound **6** (10 mg) gave a tetraacetate (**6a**) upon acetylation with $Ac_2O$–pyridine; this product was recrystallized from MeOH to give yellow needles (12 mg), mp 218 °C. UV $\lambda_{max}^{dioxane}$ nm (log ε): 264 (4.84), 285 (sh, 4.76), 335 (3.84). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 1770, 1670, 1585. High resolution MS *m/z*: Calcd for $C_{24}H_{20}O_{11}$: 484.1004. Found: 484.0987. $^1$H-NMR data are shown in Table II.

**Methylation of 6**——$Me_2SO_4$ (0.1 ml) and $K_2CO_3$ (100 mg) were added to a solution of **6** (2 mg) in $Me_2CO$ (1 ml), and the mixture was refluxed for 4 h, then treated as above.

The permethyl ether of **6** was identified as 1,2,6,7,8-pentamethoxy-3-methylanthraquinone by direct comparison with an authentic sample.

**Conversion of Aurantio-obtusin to 6**——Aurantio-obtusin (10 mg) dissolved in AcOH was treated with HBr (1 ml) and the mixture was refluxed for 10 min. The reaction mixture was refluxed for 10 min. The reaction mixture was diluted with AcOEt, washed with $H_2O$ and concentrated *in vacuo*. The residue was chromatographed on $SiO_2$ with $C_6H_6$–AcOEt (9 : 1) and the product was recrystallized from $Me_2CO$ to afford yellow-brown needles (4 mg), mp >300 °C. The crystals were identified as **6** by IR, $^1$H-NMR and MS.

**Acknowledgements** The authors are grateful to Prof. U. Sankawa, Faculty of Pharmaceutical Sciences, University of Tokyo, for providing the authentic sample of questin. Thanks are also due to Miss Y. Kimura of the Department of Pharmacy, Nihon University, for IR spectra, and Mr. M. Aimi and Dr. T. Takido of the Analytical Centre, College of Science and Technology, Nihon University, for MS and NMR spectra.

## References and Notes

1) Part XIV in the series "Studies on the Constituents of Purgative Crude Drugs." For Part XIII see S. Kitanaka and M. Takido, *Phytochemistry*, **21**, 2103 (1982).
2) M. Takido, *Chem. Pharm. Bull.*, **6**, 397 (1958).
3) M. Takido, *Chem. Pharm. Bull.*, **8**, 246 (1960).
4) M. Takido and S. Takahashi, *Shoyakugaku Zasshi*, **17**, 43 (1964).

5) Y. Kimura, M. Takido and S. Takahashi, *Yakugaku Zasshi*, **86**, 1087 (1966).
6) S. Kitanaka and M. Takido, *Phytochemistry*, **20**, 1951 (1981).
7) R. Kinget, *Planta Med.*, **15**, 233 (1967).
8) A. Mahmoodian and C. E. Stickings, *Chem. Ind.* (London), **1962**, 1718.
9) S. Shibata, M. Takido and O. Tanaka, *J. Am. Chem. Soc.*, **72**, 2789 (1950).
10) W. Steglich and W. Losel, *Tetrahedron*, **25**, 4391 (1969).
11) L. H. Briggs, G. A. Nicolls, and R. M. L. Paterson, *J. Chem. Soc.*, **1952**, 1719.