The filtrate from L-tryptophan was concentrated to dryness and the residue was dissolved in 8 cc. of water. The solution was acidified with HCl and the resulting precipitate was collected by suction. Recrystallization from 50% MeOH gave 2.6 g. (84.5%) of 1-N-acetyl-D-tryptophan, m.p. 184~185°, $[\alpha]_D^{15}$ −28°(c=2, EtOH). *Anal.* Calcd. for $C_{13}H_{14}O_3N_2$: C, 63.40; H, 5.73; N, 11.38. Found: C, 63.62; H, 5.86; N, 11.46.

(ii) To 300 cc. of 0.05*M* 1-N-acetyl-DL-tryptophan solution, 0.8 g. of KT 104 acetone-dried powder was added and the mixture was incubated at 37° for 2~3 days. 1.0 g. (65%) of L-tryptophan and 1.5 g. (81%) of 1-N-acetyl-D-tryptophan were obtained.

(iii) To 500 cc. of 0.05*M* 1-N-acetyl-DL-tryptophan solution, 40 cc. of KT 104 cell-free extract was added and the mixture was incubated at 37° for 1~2 days. 1.8 g. (70.5%) of L-tryptophan and 2.6 g. (84.5%) of 1-N-acetyl-D-tryptophan were obtained.

Summary

It was found that a strain of *Pseudomonas* sp., KT 104, isolated from the soil of a bamboo thicket in Kanazawa, has the ability to hydrolyze asymmetrically 1-N-acetyl-DL-tryptophan to give L-tryptophan and 1-N-acetyl-D-tryptophan. The strain may be used in the form of bacterial mass, acetone-dried powder, or cell-free extract.

KT 104 can be cultivated in a synthetic medium containing N-benzoylanthranilic, benzoic, *p*-hydroxybenzoic, salicylic, anisic, or phenylacetic acid as the sole source of carbon, and ammonia as the sole source of nitrogen, at 25°, for at least 3 generations.

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75. Michio Takido: Studies on the Constituents of the Seeds of *Cassia obtusifolia* L. I. The Structure of Obtusifolin.

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The seeds of *Cassia obtusifolia* L. (Leguminosae) are used as purgative and tonic, and have been employed as a yellow dyestuff in this country.

Some chemical studies have been made on the constituents of this seed. Elborne[1] and Shimoyama[2] isolated emodin, and recently Kariyone and Tsukita[3] by paper chromatographical study reported the existence of chrysophanol or physcion, chrysophanol anthrone, emodin, aloe-emodin, and rhein.

In the present work, chrysophanol (IV), physcion (VII), and a new anthraquinone derivative, to which the name obtusifolin is proposed, were isolated from the seeds by column chromatography.

Obtusifolin, yellow needles, m.p. 237~238°, whose analyses agreed with $C_{16}H_{12}O_5$ involving one methoxyl group, is insoluble in water and 5% $NaHCO_3$, but soluble in 5% Na_2CO_3 to give an orange solution.

It gives a purple-blue color in conc. H_2SO_4, brown with $FeCl_3$, and orange in alcoholic $Mg(OAc)_2$ solution. The infrared spectrum of obtusifolin indicated the presence of a free hydroxyl group and both chelated and non-chelated carbonyl. It was therefore suggested that obtusifolin is an anthraquinone derivative with at least one free hydroxyl group in the β-position.

Obtusifolin gives diacetate, light yellow needles, m.p. 187~188°; dimethyl ether,

* Surugadai, Tokyo (滝戸道夫).

1) Elborne: Pharm. J., Trans., **3**, 242(1889).
2) J. Shimoyama: Apotheker Ztg., **11**, 537(1896).
3) T. Kariyone, K. Tsukita: Yakugaku Zasshi, **74**, 223, 225(1954).

light yellow needles, m.p. 145~146°; diethyl ether, light yellow needles, m.p. 127°; and monomethyl ether, yellow needles, m.p. 172.5°. (The absence of free hydroxyl group was indicated by infrared spectrum). Norobtusifolin was obtained by treating obtusifolin with HBr or heating with conc. H_2SO_4 at 100°.

Nor-obtusifolin, $C_{15}H_{10}O_5$, orange red needles, which is soluble in 5% aq. Na_2CO_3 in purple, shows purple-blue color in conc. H_2SO_4, and dark brown with $FeCl_3$. The infrared spectrum indicated the presence of a free hydroxyl, and both chelated and non-chelated carbonyl. It gives a triacetate, m.p. 221~222°, and trimethyl ether, m.p. 145~146°, which should be obtusifolin dimethyl ether. The ultraviolet spectrum of tri-O-acetyl nor-obtusifolin resembles closely that of di-O-acetylchrysophanol.

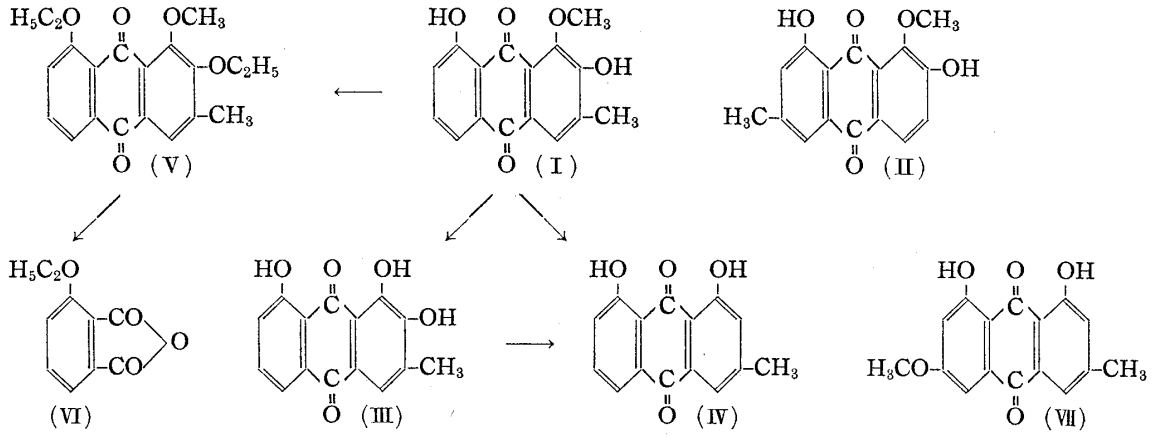
On heating with hydriodic acid and red phosphorus in glacial acetic acid, obtusifolin or nor-obtusifolin forms a reduction product which gives chrysophanol on oxidation. Thus, nor-obtusifolin (III) should be a β-hydroxylated chrysophanol. Nor-obtusifolin is a strong mordant dye, showing that it can be a derivative of alizarin. The ultraviolet spectra of nor-obtusifolin and 1,2,8-trihydroxyanthraquinone are almost superimposable.

The orange color reaction with $Mg(OAc)_2$ ruled out the possibility of the presence of 1,2-hydroxyls in obtusifolin. Therefore, the following alternative possible structures, (I) and (II), would be proposed for obtusifolin.

Finally the structural formula (I) was deduced for obtusifolin by the following observations. On oxidation of di-O-ethylobtusifolin (V) with CrO_3 in acetic acid-acetic anhydride solution, 3-ethoxyphthalic anhydride (VI) was obtained.

The dimethyl ether (m.p. 165~166°) of (II), which was already synthesized by Simonsen,[4] showed a marked difference in melting point from that of di-O-methylobtusifolin (m.p. 145~146°).

Treatment of di-O-acetylobtusifolin with CrO_3 in acetic anhydride-acetic acid solution failed to oxidize the side chain to carboxyl. As shown in cynodontin and catenarin,[5] the methyl group adjacent to hydroxyl cannot be oxidized, and the formula (I) is the case.



Thus, the structure of obtusifolin has been established as 1-methoxy-2,8-dihydroxy-3-methylanthraquinone.

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4) J.L. Simonsen: J. Chem. Soc., **125**, 721(1940).
5) W.K. Anslow, H. Raistrick: Biochem. J., **34**, 1126(1940).

of University of Tokyo. Misses T. Kurosu, M. Fukada, and N. Morita cooperated in preparing materials, to all of whom the author's thanks are due.

Experimental

Extraction of the Seeds of *Cassia obtusifolia* L. (Isolation of Chrysophanol, Physcion, and Obtusifolin)—The materials used for the present work were collected in the medicinal plant garden of Nihon University and those purchased in the market.

The seeds (1.0 kg.) were extracted three times with $CHCl_3$ (2 L.) and 20% H_2SO_4 (250 cc.), and the concentrated $CHCl_3$ extract was chromatographed on a $CaHPO_4$-column developing with benzene.

The first developed yellow band was extracted with petr. ether and chromatographed again on a $CaHPO_4$-column developing with petr. ether to give two separate bands.

From the lower band chrysophanol (yield, 200 mg.), m.p. 193~194° (from MeOH), and from the upper band, physcion (yield, 10 mg.), m.p. 206°(from MeOH), were isolated, and their identities were established by mixed fusion with authentic samples of chrysophanol (193~194°) and physcion (206~207°).

The second developed yellow band, separated long yellow needles, m.p. 237~238°, by recrystallization from MeOH, which was named obtusifolin. Yield, 200 mg. *Anal.* Calcd. for $C_{15}H_9O_4 \cdot OCH_3$ (Obtusifolin) : C, 67.60; H, 4.23; OCH_3, 10.92; mol. wt., 284. Found : C, 67.69, 67.52; H, 4.40, 4.34; OCH_3, 10.39; mol. wt. (Rast method), 291, 266. I.R. ν_{max}^{Nujol} cm^{-1} : 3302(-OH), 1656(non-chelated C=O), 1631(chelated C=O).

Diacetylobtusifolin—A mixture of obtusifolin (100 mg.) with Ac_2O and a drop of conc. H_2SO_4 or Ac_2O and pyridine was allowed to stand over night at a room temperature. The product was recrystallized from MeOH to light yellow needles, m.p. 187~188°(100 mg.). It is insoluble in 5% Na_2CO_3, gives no color reaction with $FeCl_3$ or with addition of $Mg(OAc)_2$ in EtOH. *Anal.* Calcd. for $C_{16}H_{10}O_3 \cdot (OCOCH_3)_2$: C, 65.22; H, 4.35; CH_3CO, 23.37. Found : C, 65.41; H, 4.31; CH_3CO, 23.65. I.R. $\nu_{max}^{CHCl_3}$ cm^{-1} : 1768(phenolic acetate C=O), 1678(C=O), 1698(phenyl band).

Di-O-methylobtusifolin—A mixture of obtusifolin (100 mg.), dry acetone (50 cc.), Me_2SO_4 (2.0 cc.), and anhyd. K_2CO_3 (1.5 g.), was refluxed for 8 hrs. The solvent was evaporated and the residue was heated on a water bath with 5% aq. KOH to decompose Me_2SO_4. The yellow crystals which separated were recrystallized from petr. ether to light yellow needles, m.p. 145~146° (80 mg.). It is insoluble in 5% NaOH and gives no $FeCl_3$ color reaction in EtOH. *Anal.* Calcd. for $C_{18}H_{16}O_5$: C, 69.23; H, 5.13; mol. wt., 312. Found : C, 68.88, 69.19; H, 5.00, 5.08; mol. wt.(Rast method), 327, 311.

Di-O-ethylobtusifolin—A mixture of obtusifolin (100 mg.), dry acetone (50 cc.), Et_2SO_4 (2.0 cc.), and anhyd. K_2CO_3 (1.5 g.), was refluxed for 20 hrs. The solvent was evaporated and the residue was heated on a water bath with 5% aq. KOH to decompose Et_2SO_4.

The yellow crystals which separated were recrystallized from petr. ether, to yellow needles, m.p. 127° (80 mg.). It is insoluble in 5% NaOH and gives no $FeCl_3$ color reaction in EtOH. *Anal.* Calcd. for $C_{20}H_{20}O_5$: C, 70.59; H, 5.88. Found : C, 70.36; H, 5.88.

Mono-O-methylobtusifolin—Obtusifolin (100 mg.) was methylated with CH_2N_2 (from 1.0 g. of nitrosomethylurea) in ether (30 cc.) by standing over night at room temperature. It was recrystallized from MeOH to yellow needles, m.p. 172.5°(90 mg.), which is insoluble in 5% Na_2CO_3 but soluble 5% NaOH, to give an orange solution, and gives a brown color with $FeCl_3$. *Anal.* Calcd. for $C_{17}H_{14}O_5$: C, 68.45; H, 4.70. Found : C, 68.08; H, 4.82. I.R. ν_{max}^{Nujol} cm^{-1} : 1670(non-chelated C=O), 1641(chelated C=O), 1584(phenyl band).

Nor-obtusifolin—A mixture of obtusifolin (120 mg.), glacial AcOH (20 cc.), and HBr (20 cc., 48%) was refluxed for 5 hrs. at 160~180°. The reaction mixture was poured into water to separate brown-red precipitate which was collected and recrystallized from MeOH to orange-red needles, m.p. 255° (80 mg.). It is soluble to give purple solution in 5% Na_2CO_3 and colors purple blue in conc. H_2SO_4. It gives a dark brown color with $FeCl_3$ and bluish purple with $Mg(OAc)_2$. *Anal.* Calcd. for $C_{15}H_{10}O_5$: C, 66.67; H 3.70. Found : C, 66.57, 66.50; H, 3.64, 3.52. U.V. λ_{max}^{EtOH} mμ(log ε); 424(3.64), 257(3.98), 235 (3.94). I.R. ν_{max}^{Nujol} cm^{-1} : 3362(-OH), 1657(non-chelated C=O), 1608(chelated C=O), 1555(phenyl band).

Nor-obtusifolin was also obtained by heating obtusifolin with conc. H_2SO_4 on a boiling water bath for 2 hrs.

Tri-O-acetylnor-obtusifolin—The acetate was obtained by the usual method, as used for diacetylobtusifolin, as light yellow needles, m.p. 221~222° (from MeOH). It is insoluble in 5% Na_2CO_3, gives no color with $FeCl_3$ in EtOH, and also by the addition of $Mg(OAc)_2$ in EtOH. *Anal.* Calcd. for $C_{15}H_7O_2 \cdot (OCOCH_3)_3$: C, 63.63; H, 4.03; CH_3CO, 32.82. Found : C, 63.95; H, 4.07; CH_3CO, 33.27. U.V. λ_{max}^{EtOH} mμ(log ε) : 335(3.67), 257(4.41).

Tri-O-methylnor-obtusifolin—Prepared by the usual method, giving no depression of m.p. on admixture with di-O-methylobtusifolin.

Reduction of Obtusifolin and Nor-obtusifolin (Formation of Chrysophanol)—A mixture of obtusifolin (500 mg.), red P (500 mg.), glacial AcOH (15 cc.), and HI (3 cc., sp. gr., 1.7) was refluxed for 5

hrs. The brown reaction mixture was cooled, poured into water, and decolorized with addition of a small amount of $NaHSO_3$. The resulting yellow brown precipitate was collected, washed with water, and dried. This substance gave a color reaction of anthrone (soluble in conc. H_2SO_4 in yellow brown color and separates Se from SeO_2 in conc. H_2SO_4). The crude anthrone dissolved in glacial AcOH (20 cc.) was added with a solution of CrO_3 (0.3 g.) in glacial AcOH (15 cc.). After standing for 30 mins. at a room temperature, the brown mixture was diluted with water and extracted with ether. The yellow extract freed from AcOH was shaken repeatedly with 5% Na_2CO_3, washed with water, and evaporated to dryness. The residue was recrystallized from MeOH to yellow orange needles (50 mg.), m.p. 193~194°, alone and mixed with authentic chrysophanol, m.p. 193~194°.

For further confirmation, a portion of the product was converted into the acetate, which was recrystallized from MeOH to light yellow needles, m.p. 207~208°. It showed no m.p. depression on admixture with authentic di-O-acetylchrysophanol, m.p. 208°. Chrysophanol was also obtained from nor-obtusifolin by the same method. In this case the intermediate anthrone was oxidized with 0.5% H_2O_2 in alkaline solution.

Oxidation of Di-O-ethylobtusifolin (Isolation of 3-Ethoxyphthalic Anhydride)—Di-O-ethylobtusifolin (250 mg.) was dissolved in a mixture of Ac_2O (10 cc.) and glacial AcOH (10 cc.) on a boiling water bath and, while heating, a solution of CrO_3 (1.0 g.) in water (2 drops) and glacial AcOH (5.0 cc.) was added dropwise during 30 mins., and the mixture was kept for another 30 mins. on a boiling water bath.

The reaction mixture was poured into hot water (300 cc.), then cooled, and filtered. The filtrate was extracted with ether several times, the ethereal solution was evaporated to dryness, the residue was sublimed *in vacuo*, and recrystallized from ether to colorless leaflets. The crystals were transferred into a sublimation tube and heated for 10 mins. above its m.p. It was then sublimed at 1 mm. Hg at 100° and the sublimate was recrystallized from dehyd. toluene. The product, colorless needles (10 mg.), melted at 146°, which gives no color with $FeCl_3$, gives a green fluorescent solution when it was melted with oxalic acid and resorcinol, added with aq. NaOH. It showed no depression of m.p. on admixture with an authentic synthetic specimen of 3-ethoxyphthalic anhydride, m.p. 146~146.5°.

Di-O-ethylchrysazin—Chrysazin (100 mg.) was ethylated as in the case of diethylobtusifolin to yellow leaflets (from MeOH), m.p. 173~174°. Yield, 80 mg. *Anal.* Calcd. for $C_{18}H_{16}O_4$: C, 72.97; H, 5.41. Found: C, 72.76, 72.81; H, 5.39, 5.46.

3-Ethoxyphthalic Anhydride—Di-O-ethylchrysazin (250 mg.) was oxidized as in the case of di-O-ethylobtusifolin to colorless needles, m.p. 146~146.5°. Yield, 18 mg. *Anal.* Calcd. for $C_{10}H_8O_4$: C, 62.50; H, 4.17. Found: C, 62.33; H, 4.17.

Mordant Dye Test with Nor-obtusifolin—White wool was mordanted with Ca, Zn, Sn, Cr, Al, Pb, Fe, Mg, Cu, and Cd, using $Ca(OAc)_2$, $Zn(OAc)_2$, $SnCl_2$, $K_2Cr_2O_7$, $Al_2O(OAc)_4$, $Pb(OAc)_2$, $FeCl_3$, $Mg(OAc)_2 \cdot CuO$, and $Cd(OAc)_2$ as described by Cain and Thorpe, and by Howard and Raistrick.[6] As with alizarin and 1,2,8-trihydroxyanthraquinone, nor-obtusifolin dyed wool with equal intensity and brilliance, but the color developed with Pb, Mg, Cu, Cd, Ca, and Zn was bluer in nor-obtusifolin than that in alizalin and 1,2,8-trihydroxyanthraquinone.

Summary

From the seeds of *Cassia obtusifolia* L. (Leguminosae), a new anthraquinone pigment, obtusifolin, m.p. 237~238°, was isolated along with chrysophanol and physcion. The structure of obtusifolin was established as 1-methoxy-2,8-dihydroxy-3-methylanthraquinone.

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6) B. H. Howard, H. Raistrick: *Ibid.*, **57**, 212(1954).