THE CONSTITUTION OF AWOBANIN AND AWOBANOL, THE COLOURING MATTER OF AWOBANA[1] AND ITS CO-PIGMENT.

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Flowers of "Tsuyukusa", *Commelina communis*, are beautiful azure blue and the extracted flower juice colours fabrics blue, hence the name "Tsukikusa" or "Awobana", meaning a paint grass or a blue flower. The colouring matter is one of the most well known and the oldest of its kind in Japan and the usage has a historical interest. Though no longer in practical use as a dye, as the colour can be easily washed away, it is still employed for drawing patterns in the art of "Yuzen" silk print and "Shiborizome" (tied dyeing). In some regions, therefore, the plant is especially cultivated for the production of "Awobana paper" which is painstakingly prepared by painting the flower juice on sheets of paper and drying them in the sun. No record of the chemical study on this pigment has been found.

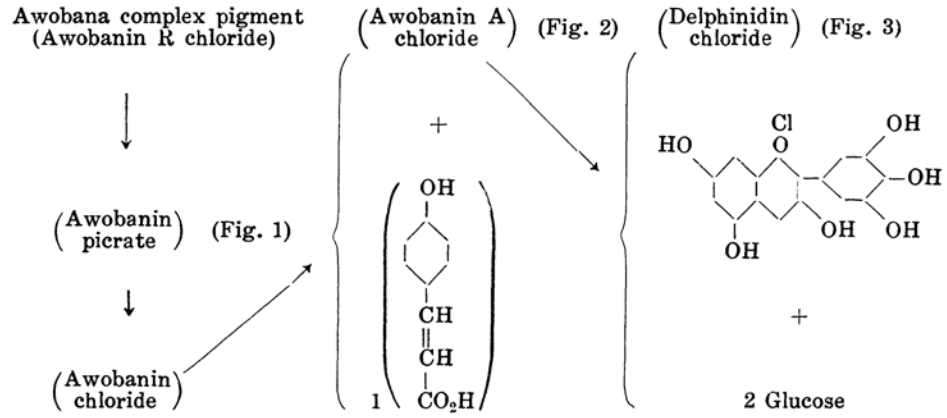
The present author was attracted to the study of this pigment, which she desired first, to obtain in a pure crystalline state, though it was difficult. However, beautiful blue coloured precipitate, like ultramarine, was finally isolated on adding methyl alcohol to its aqueous solution prepared from the Awobana paper. The blue powder thus obtained remarkably kept the colour unchanged in dry state (even in aqueous solution the colour was preserved considerably). In this respect the pigment seemed unusually stable for an anthocyanin; furthermore by decomposition with alkali, *p*-hydroxy-acetophenone which is not usually derived from anthocyanin was produced along with *p*-coumaric acid. Therefore, the constitution of this pigment was considered to belong to a special series and not to anthocyanin, and the study was followed accordingly.

However, after several investigations, it was shown that the main bright colour of this pigment is due to an anthocyanin awobanin named by the author in presence of a co-pigment awobanol also named by the author and metals which were detected in ashes after combustion (K, Mg, Al, SO_4, PO_4, etc.).

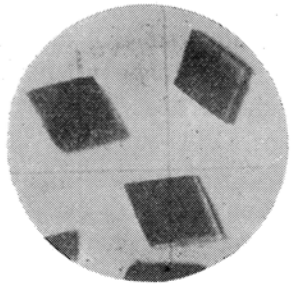
(1) "Awobana" is the local name for the flower of *Commelina communis var. hortensis* Makino used in Shiga, where the commercial Awobana paper is produced. It may not be synonymous with the so-called "Awobana" or "Tsuyukusa" (*Commelina communis*).

The above results were already reported in short notes.[2] Now, to summarise details of the above study and to report some new results, the present communication is made.

Notwithstanding difficulties in the separation of awobanin from awobanol because of their properties which resemble each other in many respects, the two were finally separated and the constitution of awobanin was successfully confirmed as follows. Awobana paper was steeped in water, to the solution, an aqueous lead acetate solution was added to precipitate the lead salt of the pigment. On treating the lead salt with CH_3OH–HCl, it was converted into the chloride (red) in methyl alcoholic solution. The chloride of the colouring matter was precipitated by the addition of ether several times, then a red powdery pigment was obtained as chloride. The isolation of pure anthocyanin from the above chloride was exceedingly difficult, however, after careful treatments, a new crystalline glucoside of the pigment awobanin was obtained as picrate (Fig. 1), and finally as chloride. Awobanin was confirmed to be a compound of another authocyanin (delphinidin 3,5-diglucoside, awobanin A named by the author) and *p*-coumaric acid by the following procedures. Analytical results of the picrate and the chloride agreed with delphinidin picrate+2 glucose+*p*-coumaric acid+$5H_2O$ and delphinidin chloride+2 glucose+*p*-coumaric acid+$5H_2O$, respectively. When awobanin was treated with cold alkali in H_2 gas, *p*-coumaric acid as one of the above component was isolated quantitatively. As another component, crystalline glucoside awobanin A finally separated slowly, when awobanin was specially treated with 20% aqueous HCl at room temperature and left for several days. The identification of awobanin A thus obtained is shown below.

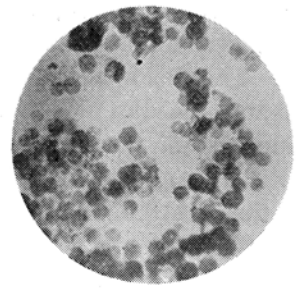


(2) Kuroda, *Proc. Imp. Acad. Japan*, **7** (1931), 61. Kuroda, *ibid.*, **9** (1933), 94. Kuroda, *ibid.*, **11** (1935), 238.



Awobanin picrate

Fig. 1.



Awobanin A chloride

Fig. 2.

Awobanin aglucone

Fig. 3.

Upon recrystallization, needle crystals were obtained from methyl alcohol and prisms from CH_3OH-HCl (Fig. 2). On analysis, both forms were found to agree with diglucoside of delphinidin chloride $C_{27}H_{31}O_{17}Cl+3H_2O$. The estimation of sugar was carried out by Pavy's method, the result being in agreement. When hydrolyzed with HCl, 1 mol of aglucone and 2 mols of sugar were produced. From the sugar, glucosazone was formed, agreeing in the properties and the melting point. The aglucone (Fig. 3) crystallized out in prisms from CH_3OH-HCl, and it gave all the reactions of delphinidin.

The analysis showed the compound to be delphinin chloride $C_{15}H_{11}O_7Cl+3H_2O$. Alkali fusion was applied to the aglucone and the glucoside. Phloroglucinol which was confirmed by the reactions and the analytical results, and also an acid which was precipitated with lead acetate were formed. The latter seems to be gallic acid by qualitative tests, etc., and was converted into trimethyl ether with dimethyl sulphate, which melted at 168° (gallic acid trimethyl ether melts at 168°). Micro-Zeisel estimation proved the absence of methoxyl groups in the aglucone.

Now the position of the glucose was confirmed to be "3" and "5" by Karrer's perhydrol method.[3] Furthermore colour reactions with Na_2CO_3 and with buffered solution were exactly in agreement with "delphinin chloride", 3,5-diglucoside of delphinidin chloride in literature.[4] Absorption spectrum was observed to be similar to the diglucoside of delphinidin chloride. The yield of the diglucoside was considerable. Delphinidin monoglucoside was also noticed.

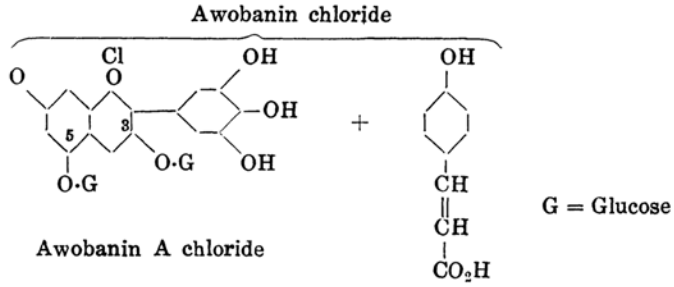
A crystalline co-pigment awobanol was obtained from the blue aqueous solution prepared from the Awobana powder, or Awobana paper, when it was heated with HCl. This compound seemed to be closely related to the chief

(3) *Helv. Chim. Acta*, **15** (1932), 1212.

(4) Reynols, Robinson, and Scott-Moncrieff, *J. Chem. Soc.*, **1934**, 1235.

pigment because of the comparatively large yield, and because of the red colouration which was shown on reduction either with Mg and HCl or with sodium amalgam in alcohol. The following studies were made upon this substance: analyses of awobanol (needles or plates) and its acetyl derivative (crystalline); acetyl determination and estimation of CH_3O-group. Alkali fusion gave *p*-hydroxy-benzoic acid and a small quantity of a product which shows the phloroglucinol reaction. Alkali decomposition also gave the above phenol, however, *p*-hydroxy-acetophenone was produced in a considerable quantity. Consequently it was assumed that awobanol is not directly related to the chief Awobana pigment and rather a co-pigment.

In concluding, the pigment in Awobana is considered to be the anthocyanin combined with *p*-coumaric acid and co-pigment awobanol.



Awobanin A chloride

The further study of the positions of *p*-coumaric acid, the co-pigment and the relations of the ashes with Awobana pigment is in progress.

Experimental.

Isolation of Awobanin. The commercial Awobana paper (800 g.) was extracted with cold water, to the resulting blue coloured solution, an aqueous lead acetate solution was added to precipitate the lead compound of the pigment; the precipitate was sucked, and dried on a tile (blue powder 100 g.). On treating the lead salt with CH_3OH–HCl (2%) it was converted into chloride in CH_3OH solution. From the resulting red solution chloride of pigment was precipitated by the addition of ether (yield 25 g.). The chloride was dissolved in absolute CH_3OH, from which the pigment was precipitated as a red powdery substance on addition of ether. Though the process was repeated several times, the pigment seemed to contain some quantity of awobanol and other impurity. Furthermore as the chloride was very easily soluble in water or CH_3OH, the isolation of the crystalline pigment from the substance was extremely difficult. The chloride was named awobanin chloride (R) by the author.

However it was finally successfully obtained in crystalline state after several experiments and especially by using the material prepared from fresh flowers, instead of Awobana paper. However, as it was generally difficult to have the material of the

Awobana pigment directly, from the fresh flowers, the author has usually studied with the material which was prepared from Awobana paper, except the above one occasion.

The fresh flowers were extracted with CH_3OH–HCl (2%) in cold; from the resulting red solution the lead salt of pigment was precipitated, and was converted into its chloride, and was purified in the usual way as mentioned above. To the solution of the chloride (R) thus obtained (7 g.) dissolved in hot H_2O (30 c.c.), picric acid (3 g.) was added, heated on the water bath, and left for one day, then an oily matter separated. When the oily matter was carefully treated with dilute aqueous picric acid on the water bath, picrate of the pigment crystallized out in reddish brown lustrous plates (2 g.). (Found for air dried material: C, 46.00, 46.06; H, 4.64, 4.68; N, 3.84, 3.93. Calculated for $C_{42}H_{39}O_{26}N_3+5H_2O$: C, 46.19; H, 4.49; N, 3.85%.) This was recrystallized from picric acid+CH_3OH (Fig. 1). The picrate was converted into chloride, when ether was added to the picrate dissolved in 2% CH_3OH–HCl, then the chloride crystallized out in red fine needles (decomposition over 200°). (Found for air dried material: C, 48.31, H, 5.54; Cl, 3.60. Calculated for $C_{36}H_{37}O_{19}Cl+5H_2O$: C, 48.1; H, 5.23; Cl, 3.8%.) This substance was easily soluble in cold water or methyl alcohol, giving a purple red colour. With sodium carbonate it gave a pure blue colour fading to clear green.

Decomposition of Awobanin with Cold Alkali. Awobanin (0.1 g.) dissolved in water (2.5 c.c.) was treated with cold aqueous NaOH (5 c.c.) in H_2 for 2 hours. The product was acidified, extracted with ether. *p*-Coumaric acid (white crystals, m. p. 208° both alone and after admixture with an authentic specimen) was isolated from the ethereal solution. (Found for material dried on H_2SO_4; C, 65.8; H, 5.0. Calculated for $C_9H_8O_3$ C, 65.9; H, 4.9%.)

When awobanin R was decomposed with hot aqueous alkali in H_2 gas, *p*-coumaric acid and *p*-hydroxy-acetophenone were isolated.

Isolation of Awobanin A. The solution of awobanin chloride (R) (1 g.) dissolved in cold 20% aqueous HCl was left for few days at room temperature; dark purple substance gradually crystallized out (0.5 g.), it was washed with CH_3OH, then with 0.5% aqueous HCl (yield 0.25 g.). For analysis a specimen was recrystallized from CH_3OH (air dry). (Found: C, 45.43: H, 5.11; Cl, 4.77. Calculated for $C_{27}H_{31}O_{17}Cl+3H_2O$: C, 45.25; H, 5.17; Cl, 4.60%.) This substance was purified under several conditions as follows: (1) From CH_3OH–HCl (2%) it crystallized in needles; (2) When it was dissolved in minimum quantity of 0.5% hot aqueous HCl and an equal volume of 3% C_2H_5OH–HCl was added, it crystallized out in plates (Fig. 2). However, analytical results were identical.

Direct Comparison of Colour Reactions of Awobanin A and Nasunin A Chloride. Both specimens were recrystallized in the same way with different results as follows. The compound was dissolved in a minimum quantity of 0.5% hot aqueous HCl and an equal volume of 3% C_2H_5OH–HCl was added. After cooling, awobanin A chloride crystallized out in small hexagonal plates with golden lustre while the other separated in fine needles with golden lustre.

The colour reactions in buffered solution of the above two specimens recrystallized in the same way were compared. The colours given by the two pigments were almost identical except that awobanin A became red at a lower *p*H than nasunin A: (1) rose-pink, fading slowly; (2) rose-pink; (3) (4) the same, fading to very pale pink rapidly; (5) (6) bluish-pink, becoming colourless rapidly; (9) (10) blue-violet; (11) violet-red; (13) violet-blue; (14) violet; (15) deep blue.

Both specimens were very sparingly soluble (or insoluble) in cold water, alcohol, and dilute aqueous HCl but readily in hot dilute aqueous HCl. All the ordinary reactions, the colours with Na_2CO_3 (a bright pure blue) and with $FeCl_3$ in alcoholic solution (violet-blue) were observed identical.

Hydrolysis of Awobanin A with Hot Acid. (1) *Isolation of delphinidin chloride (aglucone) from awobanin A.* When awobanin A (0.1 g.) was boiled with 20% aqueous HCl for three minutes, chloride of aglucone separated. This was crystallized under the same conditions with nasunidin chloride. The aglucone crystallized out in plates with a green metallic lustre which was quite identical with delphinidin chloride from nasunin A. (Found : C, 45.75; H, 4.08. Calculated for $C_{15}H_{11}O_7Cl+3H_2O$: C, 45.9; H, 4.33%.)

(2) *The colour reactions with buffered solution.* The both specimens, the aglucones from nasunin and awobanin chlorides gave quite identical results agreeing with those of delphinidin chloride in the literature: (1) red, pink in 30 seconds; (2) violet red, colourless in 30 seconds; (3) permanganate, colourless in 30 second; (4) similar to (3); (5) violet; (6) deep violet red; (9) blue violet; (10) similar to (9); (11) blue violet; (13) blue violet dichromic, pale yellow in $1^1/_2$ hours; (14) indigo blue, pale yellowish gray in $1^1/_2$ hours; (15) (16) similar to (14).

(3) *Estimation of sugar.* The above resulting 20% aqueous HCl solution (the filtrate from the aglucone) was neutralized with alkali and dried. The dry mass was treated under the same conditions with nasunin A and sugar was estimated by Pavy's method. (Found : sugar, 47.81. Calculated for $C_{27}H_{31}O_{17}Cl+3H_2O$: sugar, 50.28%.)

(4) *The position of sugar.* Awobanin A chloride (0.3 g.) was treated with 30% H_2O_2 as in the case of kuromamin (see following paper) and glucosazone was separated from position 3; m.p. 205°, 0.04 g. The filtrate from the above osazone was heated with 15% aqueous HCl on the water bath for $1^1/_2$ hours, then filtered. The filtrate was neutralized with alkali and dried in vacuum. From the residue, glucosazone was isolated by aid of $C_6H_5NH{\cdot}NH_2HCl$ and sodium acetate as usual. After purifying, yield 0.06 g.; m.p. 203° alone and in mixed sample with an authentic specimen. Micro-Zeisel estimation proved the absence of methoxyl groups in the aglucone.

Alkali Fusion of Awobanidin (Aglucone of Awobanin). Awobanidin chloride (0.8g .) was fused with NaOH (0.5 g.) and a small quantity of water at 203°, after cooling, the product was acidified with HCl, extracted with ether; the ether was evaporated, and from the ethereal residue dissolved in water, lead salt was precipitated by addition of lead acetate, the precipitate (yellowish brown) was filtered, (filtrate A). The precipitate was washed with water, then decomposed with dilute H_2SO_4 and extracted with ether; the ethereal residue dissolved in a small quantity of CH_3OH, was methylated with $(CH_3)_2SO_4$ and 50% aqueous KOH in H_2 gas; the reaction product was heated on the water bath to decompose the methyl ester and to evaporate CH_3OH, then was acidified, extracted with ether; the ethereal residue was again extracted with hot benzene. The benzene soluble part was crystallized from hot water, then gallic acid trimethyl ether, m.p. 165°, was isolated. The filtrate A was treated with dilute H_2SO_4, and $PbSO_4$ was removed; when the filtrate was extracted with ether, phloroglucinol was isolated, m.p. 208° both alone and after admixture with the authentic specimen. (Found : C, 44.5; H, 6.2. Calculated for $C_6H_3(OH)_3+2H_2O$: C, 44.45; H, 6.7%.)

Absorption Spectra of Awobanin A and Awobanidin Chloride. The substance in each case was dissolved in CH_3OH (N/10000), and iron arc was used as a light source. The photographs taken will be reproduced in another paper.

Preparation of Awobana Powder. Awobana paper was treated with water; to the resulting blue solution, CH_3OH was added, then a blue powder precipitated; this was sucked, and washed with CH_3OH well; the dry powder thus obtained was easily soluble in water giving a beautiful blue colour.

Isolation of Awobanol. The aqueous solution (or prepared directly from Awobana paper) of Awobana powder was heated with aqueous HCl (nearly 3%) for 1–2 hours, and was left. After a few days a red oily matter gradually separated and solidified. After filtering, the red powder was treated with CH_3OH, several times, then raw awobanol was obtained in pink needles. After purifying with hot CH_3OH it crystallized out in pale needles (m.p. 216°). This substance was soluble in hot CH_3OH, but insoluble in ether, and gave a brownish violet colour with $FeCl_3$. (Found: C, 57.13; H, 4.54%.) When heated with water it was converted into another yellowish flat crystals (m.p. 256°).

Preparation of Acetyl Derivative of Awobanol. (1) With sodium acetate and acetic anhydride at 100°C. a crystalline product in white needles (m.p. 148–150°) was obtained. (Found: C, 55.99; H, 4.53%.) (2) With acetic anhydride and conc. H_2SO_4 at room temperature, a crystalline product in white needles (m.p. 148–156°) was obtained. (Found: C, 55.99; H, 4.53%.)

Decomposition of Awobanol with Alkali. Awobanol was fused with NaOH in the usual way, the product was acidified, extracted with ether, from the neutral part of the ethereal residue, *p*-hydroxy-acetophenone was isolated, white needles from hot water, m.p. 107° both alone and after admixture with the authentic specimen. (Found: C, 70.23; H, 5.9. Calculated for $C_8H_8O_2$: C, 70.59; H, 5,8%.)

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