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TRANSACTIONS

Biochemical Studies on "Sotetsu" (*Cycas revoluta* Thunb.) Part V.

On the Chemical Constituents of "Sotetsu" pollen. I.

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On the chemical constituent of several pollens, from the biochemical or pharmaceutical point of view, it has been studied by many investigators ; but there is no chemical investigation as to the sotetsu pollen. The writer, therefore, has taken it on himself to investigate the biochemistry of sotetsu pollen.

From the biological point of view, the plant pollen corresponding to reproductive cell of the animal sperm, therefore arises question as to the presence of substances, which were contained in the sperm, yet had not been discovered in the vegetable kingdom. Especially in the case of sotetsu pollen, from which develops the sperm, above question is deeper than in the other cases.

That is to say, the substances in question are spermine in organic bases and protamin in proteins. The writer, therefore, to give a solution to the question experimented on the sotetsu pollen.

By some authors, hitherto, the organic bases isolated from the several pollens, are as following table :

Investigator.	Pollen source.	Organic bases isolated.
Heyl ⁽¹⁾ , ⁽²⁾	Ragweed	Adenine, Histidine, Arginine, Lysine, Agmatine, Guanosine, Betaine.
Anderson & Kulp ⁽³⁾	Corn.	Choline, <i>l</i> -proline.
Kiesel ⁽⁴⁾	<i>Pinus silvestris</i> .	Adenine, Arginine, Choline, Histidine, Guanine.

Miyake ⁽⁵⁾	Corn.	Adenine, Choline.
Vinson ⁽⁶⁾	Corn	Adenine, Arginine, Lysine, Choline.
Kiesel & Rubin ⁽⁷⁾	Sugar beet	Adenine, Betaine, Choline.

Benjamins⁽⁸⁾ reported an extract of rye pollen reacts like histamine, when tested by the method of Dale, and regularly causes contraction of guinea-pig and cat uterus; and it suggests that the presence of histamine in this pollen, and not in others investigated, indicates the special significance of rye pollen in the physiology of hay fever.

EXPERIMENTAL PART

The pollen used in this experiment are produced in Okinawa island 1935 and collected on June 20~25 in that year and immediately dried at 50°C, stored carefully in a sulphuric acid-desiccator.

I. General Composition of *Sotetsu* pollen.

The general chemical composition of sotetsu pollen is as follows:

	Sample A (Produced near Naha City.)		Sample B (Produced at Hanedi Village)	
	In 100 parts of original sample	In 100 parts of dry matter	In 100 parts of original sample	In 100 parts of dry matter
Moisture	21.87	—	14.24	—
Dry matter	78.13	100.00	85.76	100.00
Crude protein	25.74	32.94	29.00	33.81
Protein	19.09	24.44	23.50	27.38
Alcohol extract	15.29	19.57	15.80	18.42
Starch*	0	0	0	0
Dextrin	1.55	1.98	—	—
Reducing sugar (as glucose) }	0.33	0.42	0.32	0.37
Non reducing sugar (as sucrose) }	1.02	1.31	1.31	1.53
Crude fibre	24.32	31.13	26.22	30.57
Crude ash	4.54	5.81	4.77	5.56
Total N	4.12	5.27	4.64	5.41
Protein N	3.05	3.91	3.76	4.38
Non protein N	1.07	1.36	0.88	1.03
Organic base N	0.12	0.15	—	—
Ammonia N	0.04	0.05	—	—

* The sample gives no iodine test for starch.

*II. Is there Formaldehyde-containing substance
in Sotetsu pollen?*

The writer⁽⁹⁾ reported that the sotetsu seed contains fairly amount of formaldehyde-containing substance, and since it is hydrolyzed by emulsin it is probably a β -glucoside. Also on the sotetsu pollen, the reaction of formaldehyde—Rimini's, Vitali's and Yoder-Taggart's method—was experimented; but every reaction resulted the negative, in both cases with and without addition of H_2SO_4 in steam distillation.

III. Isolation and Identification of Organic Bases.

For the isolation of organic bases and protamine, 1 kg of the sotetsu pollen was extracted with ether and then with 85% alcohol at below 50°C in several times. The alcohol extract was evaporated under reduced pressure to a small volume for expelling the alcohol; then fat and lipid were removed by ether, and several impurities by Pb-acetate. The excess of Pb^{++} was removed by H_2S and the mother liquor from the Pb-sulphide was evaporated to a small volume. The concentrated solution thus obtained was diluted with 5% H_2SO_4 , and then researches were carried out on the organic bases by adding the phosphotungstic acid to above solution. The phosphotungstic acid-precipitate was decomposed by $\text{Ba}(\text{OH})_2$, and excess of Ba^{++} removed by CO_2 and the solution fractionated into five fractions.

(A) Volatile base (Ammonia).

The diluted solution of free bases obtained by above treatment was distilled under reduced pressure, and the distillate was received into dilute HCl. The hydrochloride of volatile base was insoluble in alcohol, and gave intensive Nessler's reaction.

(B) Researches of spermine by Wrede's method.⁽¹⁰⁾

After the distillation of volatile base, the concentrated residual solution was exactly neutralized with H_3PO_4 and after addition of 96% alcohol, the solution was allowed to stand for one night in ice box, but the characteristic phosphate of spermine was not formed at all.

(C) Precipitate by AgNO_3 (Adenine).

To remove the PO_4''' in the solution obtained from above experiment, the $\text{Ba}(\text{OH})_2$ was added to it, and excess of Ba^{++} removed by CO_2 and the solution of free base was slightly acidified with HNO_3 , and the precipitate prepared by AgNO_3 . From the AgNO_3 -precipitate, by decomposing with HCl, the hydrochloride of the base (0.15 g) was crystallized out. Its chloroaurate forms yellow prisms and melts at 262°C with decomposition.

Anal.: Subst.=0.1107 g; Au=0.0524 g

Found: Au=47.34%

Calc. for $(C_5H_5N_5 \cdot 2HCl \cdot 2AuCl_3 \cdot H_2O)$: Au=47.35%

The picrate of characteristic hairy needles is greenish yellow and sparingly soluble in water and it decomposes at 282°C.

(D) Precipitate by $AgNO_3$ and $Ba(OH)_2$ (Adenine).

The precipitate, prepared by $AgNO_3$ and $Ba(OH)_2$, was treated with HCl and H_2SO_4 to remove the Ag^+ and Ba^{++} . Then phosphotungstic acid was added to the above filtrate, and the precipitate was decomposed by $Ba(OH)_2$. The filtrate from Ba-phosphotungstate, after removing the excess of Ba^{++} by CO_2 , was concentrated to a small volume under reduced pressure; and then the solution of free base was neutralized with HNO_3 .

The nitrate freed from water was treated with cold absolute alcohol and the insoluble portion in absolute alcohol was converted into chloroaurate. Yield: 0.10 g. The chloroaurate forms yellow prisms and decomposes at 265~266°C.

Anal.: Subst.=0.0965 g; Au=0.0456 g

Found: Au=47.25%

Calc. for $(C_5H_5N_5 \cdot 2HCl \cdot 2AuCl_3 \cdot H_2O)$: Au=47.35%

The picrate crystallized in characteristic hairy needles, greenish yellow, sparingly soluble in water, decomposed at 282°C.

(E) Filtrate from $AgNO_3$ - and $Ba(OH)_2$ - Precipitate (Choline).

The filtrate was treated with HCl and H_2SO_4 to remove an excess of Ag^+ and Ba^{++} . Then phosphotungstic acid was added to the above solution, and the precipitate obtained thus was treated like the fraction (C). The solution of free base was converted into the hydrochloride by adding HCl, and the hydrochloride freed from water was treated with cold absolute alcohol.

The alcohol soluble portion gave a white precipitate by adding the saturated alcoholic solution of $HgCl_2$. To remove the Hg^{++} , the H_2S was passed into the solution from precipitate by $HgCl_2$. And when the mother liquor was evaporated to a small volume, the hygroscopic hydrochloride of the base was crystallized out. The chloroaurate formed yellow mossy crystals, and was sparingly soluble in water; the melting point was determined at 255°C.

Anal.: Subst.=0.2691 g; Au=0.1196 g

Found: Au=44.44%

Calc. for $(C_5H_{14}NOCl \cdot AuCl_3)$: Au=44.49%

The chloroplatinate formed orange yellow prisms, easily soluble in water,

decomposed at 237°C, and gave following analysis :

Anal. : Subst. = 0.3846 g ; Pt = 0.1205 g

Found : Pt = 31.33%

Calc. for $(C_6H_{14}NOCl)_2PtCl_4$: pt = 31.64%

The above results agree fairly with choline derivatives ; the yield of the base was 2.24 g as chloroaurate.

IV. *Researches of Protamine in Sotetsu nollen.*

The residue, after the alcohol extraction of 1 kg sotetsu pollen, was put into a bottle with 1% H_2SO_4 and shaken continuously for two hours, then sucked off, and the residue treated repeatedly in the same way until the H_2SO_4 extract caused no precipitate with about three times the volume of absolute alcohol.

The extracts were united, a large volume of absolute alcohol was added, and the vessel put in the room for 24 hours. Then filtered, washed with absolute alcohol, and finally with ether.

The pure white powder (24.60 g) obtained by above treatment, for the most part, consisted of inorganic matter and it was nearly negative even the biuret reaction.

SUMMARY

The principal results are summarized as follows :

(1) General constituent of sotetsu pollen was determined ; in the result it did not contained a starch.

(2) In sotetsu pollen, the formaldehyde-containing substance was not contained.

(3) The adenine and choline, in free state, were isolated from sotetsu pollen.

(4) An attempt at the isolations of spermine and protamine from sotetsu pollen was ended in negative results.

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Physiologically Active Crystalline Esters of Vitamin A.

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In a previous paper⁽¹⁾ K. Kawakami and the present author⁽²⁾ have reported that they have prepared crystalline compounds of fatty acid or benzoic esters of vitamin A by condensing them with maleic anhydride, but these crystals had no physiological activity and could not be regenerated into vitamin A.

The present author has now succeeded in preparing a crystalline ester of vitamin A by treating vitamin A concentrate (Biosterin) with β -naphthoyl chloride in presence of pyridine and proved to have the activity of 7200 C. L.O. units after Carr-Price test. It readily promotes the growth of albino rats and cures the xerophthalmia. Dr. S. Kato in our institute has observed that a 1/140,000 mol methyl alcoholic solution of the crystals show absorption bands at 3595, 3385, 3060, 2920, 2816 Å besides the maximum at 3225 Å.

The crystals have the formula $C_{31}H_{36}O_2$, m p 76°, it is easily hydrolysed by alcoholic potash into β -naphthoic acid, $C_{11}H_8O_2$, and pure vitamin A, the analysis of which agrees with the formula proposed by P. Karrer, i.e., $C_{20}H_{30}O$. Thus it has been definitely proved that the crystals are the ester of β -naphthoic acid and vitamin A.

EXPERIMENTAL

9 g of the vitamin A concentrate (6000 C.L.O.U.) prepared from the liver