On the Chemical Constituents of Rice-embryo.

By

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Rice-embryo constitutes only 3% by weight of the whole grain and it is very difficult to separate from it in a pure state in large quantities, so its chemical composition has not yet been studied thoroughly.

In 1912, Luigi Bernardini⁽¹⁾ reported on the analysis of the ash and the distribution of phosphoric acid in different forms in the embryo and he pointed out that the embryo is very rich in phosphoric acid of which the greater part is in the phytin-form.

M. Hamada⁽²⁾ isolated protein from the embryo, determined the distribution of amino acids in its hydrolytic products and demonstrated by feeding experiment that the protein in the embryo has a very high nutritive value. Later, Hirai⁽³⁾ investigated the ethersoluble substances of the embryo and isolated palmitic, oleic and linolic acids as the chief constituents of fats. Further, from the unsaponifiable fraction, he obtained the so-called Burian's phytosterol, besides a mixture of sterols which was readily soluble in organic solvents. From its high melting point (144°C) and low rotatory power (-23°) it was assumed by him to contain a dihydrositosterol.

Quite recently, the rice-polishing factories, Munechika and Niko & Co. have independently invented new polishing machines by which the embryo can be easily separated from the grain in an intact state and large quantities of these samples were kindly supplied by these factories to the present authors for investigation.

These samples, when carefully refined, looked quite homogeneous and were absolutely free from crushed grains, brans or other impurities. They contained in average 24% fatty matter, including about 1.2% unsaponifiable substances, besides a little waxes and a phytosterine-glucoside (phytosterolin). The waxes chiefly consisted of melissyl cerotate. Of the fatty acids, 25% were saturated and 75% unsaturated acids. From the saturated acids, palmitic, arachidic, and cerotic acids were isolated and identified; further, the presence of a little myristic and stearic acids were proved. The unsaturated acids consisted almost exclusively of oleic and linolic acids. The absence of other higher unsaturated acids was confirmed by the examination of the bromination products.

The unsaponifiable matter was treated with acetone and absolute alcohol and separated into two parts, i. e. a white amorphous powder (80%) and a

brown viscous syrup (20%). The former gave strong Liebermann reaction for sterine and the characteristic reaction for ergosterine. By repeated recrystallization from various organic solvents, some melissyl alcohol, a small amount of dihydrositosterine, stigmasterine and the so-called Anderson's γ -sitosterine⁽⁴⁾ were isolated and identified. The existence of a little ergosterine was confirmed by converting it into vitamin D by irradiation with ultraviolet rays. Further, the mother liquor of the above substances was fractionally crystallized from hexane and a new sitosterine (m. p. 156°C, $[\alpha]_D = -14.49^\circ$) was isolated. It was converted into an acetate (m. p. 111°C, $[\alpha]_D = -9.68^\circ$) and a benzoate (m. p. 129°C, $[\alpha]_D = +14.48^\circ$). This new sterine is comparatively soluble in light petroleum ether and more readily in hexane and other organic solvents. The so-called Anderson's α -sitosterine⁽⁴⁾, which was presumed by him to exist in rice polishing oil, is most probably the mixture of various sterines.

A cerebroside-like substance was also detected in the ether extract of the embryo. Probably it is the same as that obtained by W. Trier⁽⁶⁾ from rice grain. The nature of this substance will be investigated later on.

The authors have thus confirmed that the composition of the embryo oil closely resembles that of the polishing oil,

Experimental.

(I) General composition of rice-embryo.

The composition of the purified embryo, supplied from the Niko & Co., is compared with that reported by each of Sawamura and Hamada in the following table:

	Present authors	Sawamura	Hamada
Moisture	9.83	5.73	10.41
Crude protein	21.26	24.30	20.76
Protein	18.10	—	17.56
Crude fat	23.87	21.05	20.66
Fibre	3.48	9.77	10.11
N-free extract	33.76	25.68	27.82
Ash	9.09	13.47	10.24
Pentosan	5.—	—	—
Carbohydrate	21.—	13.—	—

The composition of the ash is compared with that of Bernardini⁽¹⁾ as follows:

	Present authors	Bernardini		Present authors	Bernardini
P_2O_5	5.020	6.200	MgO	1.104	1.389
SiO_2	0.370	0.250	Mn_2O_3	0.012	trace
Fe_2O_3	0.064	0.060	K_2O	2.022	1.691
CaO	0.312	0.279	Na_2O	0.196	trace

Bernardini moreover determined the distribution of phosphoric acid in the embryo and in the whole grain, with the following results:

P_2O_5 in form of	Embryo		Seed	
	In 100 parts of dry matter	In 100 parts of total P_2O_5	In 100 parts of dry matter	In 100 parts of total P_2O_5
Lecithin	0.04	0.64	0.003	0.35
Phosphatides	0.22	3.54	0.018	1.86
Phytin	5.14	82.90	0.436	45.68
Inorganic salts	0.04	0.64	trace	—
Nucleic acids	0.76	12.28	0.502	52.61
Total	6.20	100.00	0.950	100.00

The above table shows that the embryo is very rich in phosphoric acid of which the greater part is in the phytin-form.

(II) *Chemical properties of the embryo oil.*

The chemical constants of the embryo oil, extracted from the above sample with ether, are compared with the result obtained by Hirai⁽³⁾ and with that of polishing oil, reported by Takahashi⁽⁶⁾:

	Embryo oil		Polishing oil
	Present authors	Hirai	Takahashi
Acid value	26.5	29.74	14.36
Saponification value	183.0	212.19	183.54
Acetyl value	10.6	—	—
Iodine value	114.8	106.63	104.73
Hehner value	93.2	95.16	95.95
Reichert-Wollny value	0.4	2.05	0.73
Unsaponifiable matter	5.28%	4.00%	3.40%

The fatty acids obtained by saponifying the embryo oil with alcoholic potash in the usual way had the following constants:

m. p. 31~34°C; Acid value 196.3; Iodine value 109.9;
 Mean molecular weight 283; Liquid fatty acids 75%.

(III) *Phytosterolin* (*phytosterine-glucoside*).

When the ethereal extract of the rice-embryo was left standing for several hours, a brown precipitate separated out, which when recrystallized from amyl alcohol formed white needles melting at 283~290°C and gave the Liebermann's sterine reaction as well as the Molisch's reaction for sugar. From the analysis as well as from other properties, this substance was proved to be identical with the phytosterolin isolated from wheat-embryo by Nakamura and Ichiba⁽⁷⁾.

Analysis of the phytosterolin from rice-embryo :

	Substance (mg)	CO ₂ (mg)	H ₂ O (mg)	C (%)	H (%)
I	3.752	10.005	3.464	72.73	10.33
II	3.711	9.847	3.415	72.37	10.29
	Calculated for phytosterolin (C ₃₃ H ₅₅ O ₆)			72.30	10.20

Like other sterines, this substance forms an acetate melting at 167~168°C.

Analysis of the acetate :

	Substance (mg)	CO ₂ (mg)	H ₂ O (mg)	C (%)	H (%)
	4.378	11.059	3.503	68.90	8.95
	Calculated for C ₃₁ H ₅₁ O ₁₀			68.67	6.94

It forms also a benzoate melting at 198°C.

Analysis of the benzoate :

	Substance (mg)	CO ₂ (mg)	H ₂ O (mg)	C (%)	H (%)
	3.131	8.726	2.169	76.01	7.75
	Calculated for C ₆₁ H ₇₉ O ₁₀			75.90	7.52

(IV) *Separation of saturated fatty acids*.

Rice-embryo (1.5 kg) was boiled with 3.5 litres of 90% alcohol for three hours, filtered while hot and the residue was extracted three times each with 2.5 litres of 90% alcohol. The combined extract was allowed to stand overnight, whereby a flocky precipitate separated out which chiefly consisted of waxes. It was filtered off and the filtrate was evaporated in vacuum and the residue was extracted with ether. The ethereal extract was, after evaporating off the ether, saponified with alcoholic potash according to the usual method. From the mixed fatty acids thus obtained the solid saturated acids were separated by Tortelli Ruggeri's method. They had the following properties :

m. p. 60~61°C; Mean molecular weight 270; Acid value 208;
 Iodine value 5.

The solid acids were now mixed with sand and dried on a waterbath for ten days and extracted with ether. The ethereal solution was evaporated,

the residue was again dissolved in petroleum ether, filtered, and the filtrate was evaporated, whereby, 100 g saturated acids were obtained.

For the isolation of each fatty acid, the above acid mixture was converted into methyl ester and subjected to fractional distillation. For this purpose the mixture was dissolved in 220 cc methyl alcohol, treated with dry HCl gas until the solution absorbed about 8 g of the acid and after heating for two hours on a water-bath the solution was shaken with ether. The ethereal extract was, after washing with water to remove the methyl alcohol and hydrochloric acid, fractionally distilled under diminished pressure (8 mm). In this way the following fractions were obtained:

Fraction	Temperature (°C)	Yield (g)
I	160~176	1.5
II	176~186	80.5
III	186~213	7.2
IV (residue)	—	15.5

Each fraction was now saponified and free fatty acids thus regenerated had the following constants:

	Yield (g)	Acid value	Mean mol. wt.	m. p. (°C)
I	1.2	220.6	254.2	53~54
II	75.0	216.8	258.7	58~59
III	6.5	212.8	263.6	59~60
IV	12.3	—	—	—

The fraction I was recrystallized from 90% alcohol and the residue from the mother liquor, after evaporating the alcohol, was recrystallized twice from 80% alcohol. Again, the residuals from the combined mother liquors were recrystallized twice from 70% alcohol. Then, evaporating off the combined mother liquors, the residual was dissolved in hot 70% alcohol, decolorized with animal charcoal and allowed to stand overnight. Thereupon, a crystalline precipitate separated out which melted at 54°C and had the mean molecular weight 234. Most probably it was myristic acid containing some impurities.

The fraction II, when recrystallized four times from 95% alcohol, melted at 62~63°C and had the acid value 218.8 and the mean molecular weight 256.3. The analysis gave the following results:

	Substance (mg)	CO ₂ (mg)	H ₂ O (mg)	C (%)	H (%)
I	4.315	11.900	4.853	75.22	12.58
II	4.001	11.000	4.487	74.98	12.55
	Calculated for C ₁₆ H ₃₂ O ₂			74.92	12.59

The analysis shows that it was palmitic acid.

The fraction III was coloured slightly yellowish, so it was dissolved in alcohol and after being decolorized with animal charcoal, recrystallized fifteen times from absolute alcohol. The crystals thus obtained melted at $59\sim 61^{\circ}\text{C}$ and had the acid value 197.8 and the mean molecular weight 282. It was proved to be a mixture of stearic and palmitic acids, but the yield was too small for further purification.

The fraction IV was dissolved in 500 cc absolute alcohol and allowed to stand for 48 hours. The precipitate formed thereby was collected and washed with absolute alcohol: the yield, 3.4 g. After recrystallization from absolute alcohol eight times more, the crystals obtained melted at $76.5\sim 77.5^{\circ}\text{C}$ with the acid value 141.1 and the mean molecular weight 398.2. The analysis gave the following results:

	Substance (mg)	CO ₂ (mg)	H ₂ O (mg)	C (%)	H (%)
I	3.903	11.209	4.533	78.33	12.99
II	3.587	10.301	4.250	78.33	13.25
	Calculated for C ₂₆ H ₅₂ O ₂			78.61	13.13

The analysis shows the agreement with cerotic acid. The filtrates of cerotic acid were united, evaporated and the residue was recrystallized three times from absolute alcohol. The fatty acid obtained in this way had the melting point 76°C and the mean molecular weight 318. From this it was concluded that arachidic acid was present; but it could not be purified.

The mother liquor of fraction IV was diluted to 90% alcohol, the precipitate formed thereby was dissolved by heating and allowed to stand for three days, when the precipitate was collected, recrystallized from 90% alcohol and the filtrate therefrom was evaporated and the residue was recrystallized from 90% alcohol five times. The fatty acid thus obtained was long needles melting at $79.5\sim 80^{\circ}\text{C}$ with the acid value 164 and the mean molecular weight 342.

Analysis of the fatty acid:

	Substance (mg)	CO ₂ (mg)	H ₂ O (mg)	C (%)	H (%)
I	4.374	12.429	5.121	77.50	13.10
II	4.942	14.031	5.716	77.43	12.94
	Calculated for C ₂₂ H ₄₄ O ₂			77.57	13.03

The analysis shows the substance to agree with behenic acid.

(V) Bromination of unsaturated fatty acids.

Unsaturated fatty acids (150 g) were brominated in usual way and the bromination products were digested with 2 L of boiling petroleum ether ($35\sim 55^{\circ}\text{C}$), whereby a clear solution was obtained leaving no insoluble residue.

On standing overnight, 73 g of solid bromides separated out. The mother liquor, when concentrated to 1/3 of its volume and allowed to stand for three weeks, gave 3 g more of solid bromides.

When the solid bromide thus obtained was recrystallized three times from 95% alcohol, it melted at 113°C and had the acid value 93.7 and the mean molecular weight 599.9.

Analysis of the solid bromide :

	Substance (mg)	AgBr (mg)	Br (%)
I	6.645	8.295	53.12
II	6.030	7.553	53.30
	Calculated for $C_{13}H_{32}O_2Br_4$		53.33

The analysis shows it to agree with the tetrabromide of linolic acid.

When the filtrate of the solid bromide was evaporated, another bromide having 41.3% bromine was obtained. Probably it was the dibromide of oleic acid mixed with some tetrabromide.

(VI) *Oxidation products of the unsaturated fatty acids.*

When the unsaturated fatty acids were oxidized with alkaline permanganate according to the Hazura's method, 11.3 g insoluble hydroxyacids were obtained. They were treated with ether and separated into the ether-soluble and insoluble parts. The former, when recrystallized from absolute alcohol, melted at 131°C with the acid value 176.3 and the mean molecular weight 316.7. This was proved to be dihydroxystearic acid.

Analysis of the dihydroxystearic acid :

Substance (g)	CO ₂ (g)	H ₂ O (g)	C (%)	H (%)
0.6982	0.2462	0.09852	68.35	11.14
	Calculated for $C_{18}H_{36}O_4$		68.29	11.47

The ether-insoluble part, when recrystallized from absolute alcohol, melted at 173°C, its acid value being 160.9 and the mean molecular weight, 394.1. It was sativic acid.

Analysis of the sativic acid :

	Substance (mg)	CO ₂ (mg)	H ₂ O (mg)	C (%)	H (%)
I	3.352	7.620	3.036	62.00	10.13
II	3.611	8.178	3.391	62.07	10.35
	Calculated for $C_{18}H_{36}O_6$			62.07	10.35

Linusic or isolinusic acid could not be detected.

(VII) *Unsaponifiable matter.*

When 20 kg rice-embryo was extracted with benzene, about 4 kg dark brown oil was obtained. It was dissolved in 12 L ether, a little insoluble waxy substance was filtered off and evaporated. The oil thus obtained was saponified with alcoholic potash in the usual way and 150 g of an unsaponifiable

substance were obtained. It was a light brown semi-solid mass having a characteristic odour and giving a strong Liebermann-Buchard's reaction as well as the colour reaction of ergosterine. By treating with acetone and absolute alcohol it was separated into two part, i. e. (A) a white amorphous solid (120 g) and (B) a brown syrup (30 g).

(A) From the spectrographic studies it was calculated that the amorphous solid contained about 0.4~0.5% ergosterine. When irradiated with ultra-violet rays and tested on rats, the existence of vitamin D was clearly demonstrated.

(1) Melissyl alcohol.

When the amorphous solid (A), mentioned above, was recrystallized several times from absolute alcohol until it gave no more Liebermann-Buchard's reaction, about 0.05 g crystals was obtained which melted at 83°C.

Substance (mg)	CO ₂ (mg)	H ₂ O (mg)	C (%)	H (%)
2.798	6.462	3.524	82.48	14.09
	Calculated for C ₃₀ H ₆₂ O		82.19	14.15

The analysis shows it to agree with melissyl alcohol.

(2) Dihydrositosterol.

The mother liquors of the last few fraction of the said recrystallization were combined and evaporated. About 30 g of the residue thus obtained were again repeatedly recrystallized from absolute alcohol, and after drying, it weighed 3.2 g; m. p. 132°C, $[\alpha]_D^{25} = -6.20^\circ$. The unsaturated sterines were decomposed by the Anderson's method, and about 0.13 g dihydrositosterine (m. p. 144°C, $[\alpha]_D^{25} = +24.28^\circ$) was isolated and identified.

The sterol obtained from the combined filtrate of the above recrystallization melted at 138~140°C, $[\alpha]_D^{25} = -26^\circ$. This was extracted three times with a large quantity of petroleum ether (50°C) and then it was converted into acetate; m. p. 115~11°C, $[\alpha]_D^{25} = -23.98^\circ$.

(3) Tetrabromide of stigmastierine acetate.

When the acetate, mentioned above, was recrystallized ten times from absolute alcohol, the top fraction (12 g) melted at 138°C and had the rotation, $[\alpha]_D^{25} = -43.7^\circ$. This fraction was brominated by the Windaus-Hauth method adding an excess of glacial acetic acid. The precipitate was collected on a filter, dissolved again in chloroform and thrown down by methyl alcohol; m. p. 204~205°C.

Analysis of the bromide:

	Substance (mg)	AgBr (mg)	Br (%)
I	5.235	4.970	40.40
II	8.330	8.030	41.00
	Calculated for C ₃₂ H ₅₂ O ₂ Br ₅		40.58

The above result agrees with the tetrabromide of stigmastierine acetate.

Owing to the scarcity of the material, the free stigmastierine could not be obtained.

(4) Anderson's γ -sitosterine⁽⁴⁾.

The bromides in the mother liquor of the above tetrabromide were combined, dissolved in ether and fractionally precipitated with methyl alcohol. The main fraction thus obtained was an amorphous powder, melting at 130°C. It was debrominated by boiling with zinc dust and acetic acid, and an acetyl derivative (m. p. 143°C, $[\alpha]_D^{20} = -44.70^\circ$) was prepared. By saponifying the latter, a free sterol (m. p. 148°C, $[\alpha]_D^{21} = -42.3^\circ$) was isolated.

Analysis of the free sterol:

Substance (mg)	CO ₂ (mg)	H ₂ O (mg)	C (%)	H (%)
4.013	12.270	3.950	83.39	12.30
	Calculated for C ₂₉ H ₅₀ O		83.96	12.24

The analysis shows that it agrees with Anderson's γ -sitosterol.

(5) A new sitosterol.

The sterol acetates described above were combined and saponified. About 80 g free sterol thus obtained, was dissolved in hexane and fractionally precipitated by gradual cooling as follows:

	Temperature (°C)	Yield (g)	m. p. (°C)	D
I	21	19.5	135	-29.34° (21°C)
II	1	16.5	134	-23.93° (20°C)
III	-12	20.0	132	-18.46° (18°C)
IV (residue)		22.0	148	—

The author could not separate from the above fraction I, II, III any substance that had constant melting point and rotation. The last fraction (IV) was recrystallized from absolute alcohol ten times, from acetone twice and further from methyl alcohol fifteen times and a new sitosterol (m. p. 156°C, $[\alpha]_D^{23} = -14.485^\circ$) was isolated; yield, 2.1 g. It was more readily soluble in organic solvents than other sterines.

Analysis of the new sitosterol:

	Substance (mg)	CO ₂ (mg)	H ₂ O (mg)	C (%)	H (%)
I	3.269	10.040	3.495	83.76	11.96
II	4.144	12.690	4.510	83.52	12.18
	Calculated for C ₂₇ H ₄₆ O			83.85	12.01

The acetate of the new sitosterol was prepared by boiling the free sterol with acetic anhydride for 40 minutes in the usual way; m. p. 111°C, $[\alpha]_D^{23} = -9.87^\circ$.

Analysis of the acetate:

	Substance (mg)	CO ₂ (mg)	H ₂ O (mg)	C (%)	H (%)
I	3.472	10.370	3.608	81.46	11.63
II	3.849	11.467	3.949	81.25	11.48
	Calculated for C ₂₉ H ₄₈ O ₂			81.21	11.30

The propionate was obtained by boiling the free sterol with propionic anhydride for 1.5 hours in the usual way; m. p. 106°C, $[\alpha]_D^{25} = -6.78^\circ$

Analysis of the propionate:

	Substance (mg)	CO ₂ (mg)	H ₂ O (mg)	C (%)	H (%)
I	3.003	8.992	3.177	81.66	11.84
II	3.610	10.800	3.750	81.59	11.62
	Calculated for C ₃₀ H ₅₀ O ₂			81.74	11.60

The benzoate was obtained by adding benzoylchloride to the sterol dissolved in pyridine at 2°C; m. p. 129°C, $[\alpha]_D^{25} = +14.48^\circ$

Analysis of the benzoate:

	Substance (mg)	CO ₂ (mg)	H ₂ O (mg)	C (%)	H (%)
I	2.981	9.071	2.885	82.99	10.83
II	3.660	11.178	3.577	83.29	10.93
	Calculated for C ₃₄ H ₅₄ O ₂			83.21	10.28

The acetate mentioned above was brominated by the Windaus-Hauth method, but when a small amount of bromine was added, the solution became brownish green, liberating HBr and required 50% more bromine than the calculated quantity. When bromine was added at -13°C , the green colour never appeared, only turning brown. The percentage of bromine was found to be 31.89 and 32.27%. A portion of the sterol was reduced with hydrogen using platinum-black as the catalyst, but the reduction proceeded very slowly, so that the Liebermann-Buchard reaction never disappeared. The molecular formula of the sterol calculated from its analytical results corresponds to C₂₇H₄₆O.

From the above observation it was believed to be an isomer of Burian's sitosterol. The so-called Anderson's α -sitosterol was still a mixture of various sterols, as is shown in the following table:

	Free sterol		Acetate	
	m. p. ($^\circ\text{C}$)	Rotation	m. p. ($^\circ\text{C}$)	Rotation
α -Sitosterol	138~140	-23.41°	115~116	-23.91°
Author's new sterol	156	-14.485°	111	-9.87°

(B) The brown syrup.

The brown syrup was subjected to steam distillation and a little oil having a characteristic odour was obtained, but the amount was so small that it could not be examined further. From the residue, when further distilled

at high vacuum a hydrocarbon mixed with a little higher alcohol was obtained.

(VIII) *Wax.*

When rice-embryo was boiled with strong alcohol or benzene, then filtered while hot and the filtrate was left to stand for several hours, the waxy substance separated out as a greyish white flocky precipitate, or when the crude embryo oil was treated with ether, the waxy substance remained as an insoluble residue. These were combined and extracted with hot benzene in Soxhlet apparatus and the extract was precipitated with ether. By repeating this operation five times needle-shaped crystals melting at 82°C were obtained; yield, 3.2 g.

The analysis gave the following results:

	Substance (mg)	CO ₂ (mg)	H ₂ O (mg)	C (%)	H (%)
I	4.000	12.055	4.954	82.19	13.86
II	3.840	11.642	4.858	82.17	14.07
	Calculated for C ₆₅ H ₁₁₂ O ₂			82.06	14.03

The analysis shows that it was melissyl cerotate.

For further identification, this substance was saponified by boiling with 5% potassium alcoholate for 4 hours. After cooling, the mixture was treated with alcoholic CaCl₂. The calcium soap thus formed was filtered off, and the filtrate was completely dried and extracted with benzene. The benzene solution was evaporated and the residue was recrystallized from absolute alcohol. In this way hair-like needles melting at 85°C were obtained.

Analysis of the sample:

	Substance (mg)	CO ₂ (mg)	H ₂ O (mg)	C (%)	H (%)
	4.100	12.400	5.230	82.48	14.27
	Calculated for C ₈₀ H ₁₆₂ O			82.08	14.28

The result agrees with melissyl alcohol.

The Ca-soap obtained as above, was decomposed with dilute hydrochloric acid (1:1) and cerotic acid was isolated as insoluble crystalline powder. After recrystallization from absolute alcohol, it melted at 78°C and had the mean molecular weight 394. Mixed with a pure specimen of cerotic acid, no depression of melting point was observed.

(XI) *Cerebroside.*

When rice-embryo, previously extracted with benzol, was boiled with 85 ~90% methyl alcohol and the methyl alcoholic solution was evaporated in vacuum to a syrupy consistency and left standing for several days, a solid substance separated out. It was collected by suction and dissolved in ether. The ethereal solution thus obtained was, after washing with water, treated with acetone, whereby a precipitate was formed which gave ninhydrine and

Molisch's reactions. This precipitate was dissolved in ether and precipitated by adding absolute alcohol. By repeating this operation four times, a crystalline substance was obtained which gave no more the above reactions. When dried in vacuum desiccator it became brownish black and was no more soluble in ether. The mother liquor of the above crystals was shaken with water and the separated ether layer was dehydrated, evaporated to a small volume and treated with acetone, whereby a precipitate was formed, which when recrystallized from absolute alcohol gave ninhydrine and Molisch's reactions. It resembled the cerebroside, obtained by Trier from rice grain. Owing to the scarcity of the material, it was not further investigated. Details concerning this part will be published later.

In conclusion, the authors wish to express their sincere thanks to Prof. Dr. U. Suzuki for his advices and encouragement throughout this work and also to Mr. T. Iki and T. Tanaka for micro-analysis.

Studies on the Fermentation products by Mould Fungi. Part X.

Glaucic Acid formed by *Aspergillus glaucus*.
(*Aspergillus glaucus*. IV.)

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On the previous report of this series (part IV), the author described that a new crystal substance, m. pt. $238\sim 9^{\circ}$, was obtained by the cultivation of *Aspergillus glaucus* under a certain condition. Since, the author cultivated *Aspergillus glaucus* several times under the same condition but could not obtain this crystal substance. Unexpectedly, the author obtained this substance by treating the mycelium of *Aspergillus glaucus* with ether on account of the isolation of chitin from the mycelium. This new crystal substance was named glaucic acid after *Aspergillus glaucus*.

Glaucic acid melts at $238\sim 9^{\circ}$ and contains no nitrogen, methoxyl, ketomethylen and methylenoxide groups. The results of analyses of glaucic acid and its derivative show that the molecular formula of glaucic acid is $C_{17}H_{22}O_5$.