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On the Hydrolytic Enzyme in the Resting Seeds of Some Gramineæ.

By

Yoshio Tanaka, *Kogakushi.*

It has been clearly recognised by several investigators that the diastase of raw grain is differentiated from that of germinated grain by its inability to liquefy starch paste, and by the fact that, whilst the optimum temperature of the action of the latter lies between 50° and 55° C., that of the former is between 45° and 50°C.

The conversion product of the action of ungerminated barley diastase on starch has recently been examined by J. L. Baker.* According to this author, the liquefaction of 3 per cent. starch paste is effected in about 3 hours by the use of diastase prepared from a quantity of barley equal to that of starch used; also, at the end of 20 hours the reducing power of the solution is $R_{3.93}$ 60.11 and the iodine reaction is still blue. He also investigated the action of barley diastase on soluble starch at 50°C. and found that the saccharification proceeded very rapidly, and that the hydrolysis was practically complete in 2 hours, the reducing power being R 60 to 65 at the end of that time. The iodine reaction of the solution was still blue at 24 hours, and violet at 96 hours. Baker obtained, as the conversion product, maltose and a dextrin which gave a blue colouration with iodine and was hydrolysed very

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* J. L. Baker, Jour. Chem. Soc., 1902, p 1177—p 1185.

slowly by barley diastase, producing maltose and a small quantity of glucose. He has proposed to call this dextrin *α-amylodextrin.*

The writer has recently met with a diastase in the ungerminated seeds of glutinous millet (Japanese: *mochi-awa*)—Setaria italica *Kth*. The diastase differs markedly from that of ungerminated barley. Its hydrolytic action takes place most energetically at 50°—55° C., while the optimum temperature for barley diastase is 45°—50°C. It is capable of liquefying 3 per cent. starch paste in 20—30 minutes using diastase prepared from a quantity of glutinous millet equal to that of starch used, while in the case of barley diastase the liquefaction of starch paste occurs in about 3 hours. This diastase differs moreover from barley diastase in carrying the hydrolysis of starch paste further until no more iodine colouration is produced, the final product being maltose and achroodextrin. The diastase has a strong liquefying power and therefore it saccharifies starch paste and soluble starch with equal rapidity.

In the intermediate stages of the hydrolysis, a dextrin was isolated which was coloured red by iodine and very slowly hydrolysed by millet diastase. This dextrin is probably a special kind of erythrodextrin. Erythrodextrin and achroodextrin were also detected in the intermediate product. I also investigated the action of millet diastase on the starches of glutinous variety* which are coloured red by iodine solution. In these cases the liquefaction of the paste took place more readily than in the case of potato starch paste but the saccharification proceeded more slowly.

The same diastase can also be extracted from other cereals having small grains, that is, Japanese *kibi*—Panicum miliaceum

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* Y. Tanaka, this jour., Vol. IV, No. 2, 15—29.

L., Common millet (Japanese: *uruchi-awa*), etc., but since the investigations of the diastase of glutinous millet have been more fully carried out I shall describe chiefly the results obtained with the latter.

Experimental.

The Optimum Temperature.

3·6 grams. of an air-dried potato starch (3·0 grams. as dry matter) were gelatinised with 75 c.c. of water and hydrolysed for 7 hours with 25 c.c. of millet extract (extract from 5 grams. of glutinous millet), working at temperatures increasing from 45° to 65° C. The resulting solutions were boiled and in each case the volume after cooling was made up to 100 c.c. and tested. The comparative results may be seen in the following table:—

Temperature.	Number of c.c. of the saccharified solution required to reduce 20 c.c. of Fehling solution.
45°	7·5
50°	6·7
55°	6·4
60°	8·6
65°	14·5

This shows that the optimum temperature of the action of the diastase is 50°—55°C., the temperature being higher than that of translocation diastase and equal to that of secretion diastase. Several repetitions of the experiment confirmed the above results.

Action of Millet Diastase on Starch Paste.

(a). Saccharifying and Liquefying Powers of the Diastase.

The following experiment was undertaken to obtain some knowledge of the nature of millet diastase.

About 60 grams. of pure potato starch were mixed with 300 c.c. of water and then gelatinised with 1700 c.c. of boiling water. The starch paste was heated on the water bath to ensure complete gelatinisation. After cooling it to 58 C., millet diastase solution prepared by Lintner's method from 50 grams. of glutinous millet was added, by which the temperature was lowered to about 55°C. At this temperature the saccharification was carried out and the maltose produced was determined at intervals; the solid matters in 100 c.c. of the saccharified solution, calculated from solution density, varied from 2·75 grams. to 2·80 grams. The result was as follows:—

Time of hydrolysis in hours.	Grams. of maltose in 100 c.c. of saccharified solution.	Iodine reaction.	
		Reaction with a small quantity of iodine solution.	Reaction with excess of iodine solution.
1	0·25	Blue	Bluish Violet
2	0·35	,,	,,
3	0·50	,,	Red
4	0·70	,,	,,
5	0·88	Violet	,,
6	1·05	Red	,,
7	1·19	,,	,,
8	1·30	Pink	,,
9	1·46	,,	Pink
10	1·60	,,	,,
11	1·72	None	None

In this experiment the starch paste was completely liquefied in about 30 minutes. According to Baker's investigations barley diastase hydrolyses starch paste with ease and a reducing power $R_{3.93}$ of 38·70 is reached in two hours, but the liquefaction of paste occurs in 3 hours and even at the end of 20 hours, the iodine reaction is blue. My experiment which was carried out in conditions analogous to Baker's showed:

That the saccharifying power of millet diastase is weaker than that of barley diastase, but that the liquefying power of the former is considerably stronger than that of the latter;

That α-amylodextrin which was proposed by Baker as the name of the dextrin produced by the action of barley diastase on starch and which gives a pure blue colouration with iodine and exhibits resistance to the action of barley diastase, is not probably produced in the case of millet diastase, because a body giving blue iodine colouration is rapidly converted into dextrins which are coloured red by iodine and the blue iodine colouration completely vanishes in 4—5 hours;

That millet diastase readily converts starch paste and soluble starch chiefly to a dextrin which is coloured red by iodine and is very slowly hydrolysed by millet diastase.

(b). Isolation of the Substances formed by Hydrolysis.

In order to isolate the bodies produced, the hydrolysis of starch paste by the precipitated diastase of glutinous millet was carried out in precisely the same way as in the foregoing experiment. The hydrolysis was allowed to proceed until the iodine reaction had completely vanished; the resulting solution was then evaporated to a thin syrup, and boiled with one litre of 95 per cent. alcohol for 30 minutes under a reflux condenser. The separated dextrin was dissolved in a small quantity of water and reprecipitated with 95 per cent. alcohol; the same operation was repeated several times.

The dextrin thus prepared was readily soluble in water and not precipitated by baryta water; its aqueous solution gave no colour with iodine and it had a specific rotatory power of $[\alpha]_{D3.95}=+202{\cdot}8$, and a very slight reducing power. These results clearly show that the substance is achroodextrin. From the alcoholic solution, a sugar was obtained which had a specific rotatory power of $[\alpha]_{D3{\cdot}95}=+139{\cdot}2$ at 20°C. and a reducing power of $R_{3{\cdot}95}=98{\cdot}99$. When it was treated with phenylhydrazine acetate, phenylmaltosazone was formed which separated from the cold solution in the yellow crystalline form. The sugar under examination was consequently maltose. From these facts, it is very manifest that the final products of the hydrolysis wholly consist of maltose and achroodextrin.

Another attempt was made to isolate bodies produced in the intermediate stages of hydrolysis. For this purpose the duration of hydrolysis was limited to 6 hours, at the end of which time the conversion product showed a pure red colouration with iodine. The saccharine liquid was evaporated to a syrup, poured into a large quantity of hot alcohol and boiled for 30 minutes under a reflux condenser. From the alcoholic solution I obtained a sugar having a specific rotatory power of $[\alpha]_{10{\cdot}95}=+137{\cdot}36$ and a reducing power of $R_{3{\cdot}95}=99{\cdot}2$. It yielded a pure maltosazone. Thus the sugar formed during the hydrolysis was wholly maltose.

The precipitated dextrin was further purified by successive treatments with alcohol and dried over sulphuric acid. The dextrin was obtained as a white amorphous powder, readily soluble in hot water, and its aqueous solution gave a pure red colouration with iodine. It had a specific rotatory power varying between $[\alpha]_{D3{\cdot}95}=+199{\cdot}6—+201{\cdot}5$, and a reducing power of $R_{3{\cdot}95}=1{\cdot}25$. From these results the dextrin appears to be erythrodextrin, but the peculiarities that the greater part of the dextrin was readily precipitated by a small quantity of baryta water and that the dextrin was hydrolysed by millet diastase more slowly than the erythrodextrin, show that the essential part of the dextrin is probably not erythrodextrin, although a small quantity of the latter seems to be contained in it. Achroodextrin was also detected in the above precipitated dextrin. To an aqueous solution of the above dextrin was added a large excess of saturated baryta water, by which the dextrin producing iodine colouration was entirely precipitated. The clear solution obtained by filtration gave a white turbidity on the addition of a proper quantity of alcohol, which suggests the presence of achroodextrin

From these investigations, it will be seen that, the intermediate product of the hydrolysis contains achroodextrin, erythrodextrin and another dextrin which is coloured red by iodine and is probably more complex than erythrodextrin. For the sake of convenience, the name of *α-erythrodextrin* is proposed by the writer for this new dextrin.

α-Erythrodextrin.

As has been pointed out above, the crude α-erythrodextrin, isolated from the conversion product after 6 hour's saccharification, contains erythrodextrin and achroodextrin in small quantities. Since the separation of α-erythrodextrin from these dextrins in the pure state was troublesome, I was obliged to carry out my investigations with the crude α-erythrodextrin.

At first, the respective behaviours of crude α-erythrodextrin and erythrodextrin towards baryta water were determined according to Moreau's instructions[1]. Two c.c. of the one per cent. solution of each of the dextrins were mixed with the calculated amounts of water and of the saturated baryta water (sp. gr. 1·027) in corked test tubes, the volume of the mixture in each tube being 10 c.c. The mixture was allowed to stand for 24 hours and the supernatant clear solution was examined with iodine solution after acidifying with acetic acid. The result was as follows:—

Dextrin solution. c.c.	Water. c.c.	Baryta water. c.c.	Crude α-erythrodextrin.		Erythrodextrin.	
			State of mixture after 24 hours.	Iodine reactions of acidifyed filtrates.	State of mixture after 24 hours.	Iodine reactions of acidifyed filtrates.
(1) 2	7·0	1·0	Very slight ppt.	Red	Very slight ppt.	Red
(2) 2	6·0	2·0	ppt.	Pink	A little ppt.	"
(3) 2	5·6	2·4	"	"	ppt.	"
(4) 2	5·0	3·0	"	"	"	"
(5) 2	4·6	3·4	"	"	"	Pink
(6) 2	4·0	4·0	"	"	"	"
(7) 2	3·6	4·4	"	"	"	"

The apparent quantities of precipitates in (2)—(7) in the case of α-erythrodextrin are nearly equal and the differences of the iodine red colourations of the supernatant solutions contained in (2)—(7) are hardly recognisable; while in the case of erythrodextrin, the quantities of the precipitates increase from (2) to (7)

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[1] D. Moreau, Wochenschrift für Brauerei, Nr. 3 & Nr. 4, 1905.

gradually and the iodine red colouration decreases in intensity from (2) to (7). From these it may be supposed that the greater part of crude α-erythrodextrin is precipitated by 2 c.c. of baryta water, while that of erythrodextrin by 3·4. c.c. or more of the same reagent, and that α-erythrodextrin therefore is not identical with erythrodextrin, but is a substance having probably a greater molecular weight than the latter. It is, however, probable from the above table that the crude α-erythrodextrin contains a small quantity of common erythrodextrin, and that the quantity of the latter increases as the hydrolysis proceeds.

Further, I tried to compare the actions of millet diastase on α-erythrodextrin and erythrodextrin. The results may be seen from the following table which shows the hydrolytic products of 100 c.c. of 5 per cent. dextrin solution by the diastase precipitated from 6 grams. of glutinous millet at 50°—55°C. respectively:—

Time of hydrolysis in hours.	Crude α-erythrodextrin.		Erythrodextrin.	
	Maltose produced.	Iodine reaction.	Maltose produced.	Iodine reaction.
1·0	—	Red.	—	Pink
1·5	—	"	—	Slight Pink
2·0	—	"	1·41 grs.	No reaction
3·0	—	Pink	—	"
3·5	0·88 gr.	"	—	"

Thus, α-erythrodextrin exhibits a much greater resistance to the action of millet diastase than erythrodextrin.

Action of Millet Diastase on Soluble Starch.

Sixty grams, reckoned as dry substance, of soluble potato starch were dissolved in 2000 c.c. of boiling water and after cooling the solution to 55°C., the precipitated diastase from 50 grms. of glutinous millet was added and the whole was then kept at a temperature of 50° to 55°C. The following table shows the quantities of maltose determined at intervals:—

Time of hydrolysis in hours.	Grms. of maltose in 100 c.c. of saccharified solution.	Iodine reaction.	
		Reaction with very small quantity of iodine solution.	Reaction with excess of iodine solution.
1	0·29	Blue	Violet
2	0·41	,,	Red
3	0·56	,,	,,
4	0·72	,,	,,
5	0·85	Violet	,,
6	1·00	Red	,,

The iodine reactions completely vanished in 10—11 hours when the saccharified solution contained 1·71 grms. of maltose in 100 c.c. of the solution. The comparison of this table with that in the case of potato starch paste shows no marked difference between them. The identity of the hydrolytic actions of millet diastase on starch paste and soluble starch was further ascertained by using millet extract instead of precipitated diastase. Six grms. each of potato starch and soluble potato starch were boiled with 200 c.c. of water respectively. The mixture was then cooled to

55°C. and the necessary quantity of millet extract (40 grms. of glutinous millet in 200 c.c. of water) was added and saccharified at 50°—55°C. for 90 minutes. The following table gives the comparative results of the experiment, necessary corrections being made for the reducing power of the millet extract:—

Starch.	Extract used.	Maltose produced.
Starch paste	15·0 c.c.	0·73 grms.
Soluble Starch	15·0 c.c.	0·78 ,,
Starch paste	30·0 c.c.	1·35 ,,
Soluble Starch	30·0 c.c.	1·41 ,,

I have found, on examination, that the hydrolysis product of soluble starch by millet diastase is quite the same as that in the case of starch paste.

The equality of the action of the millet diastase on both starch paste and soluble starch is an interesting fact which is not met with in barley diastase at all, and is probably the necessary consequence of the nature of millet diastase which has a strong power of liquefaction and a weak power of saccharification.

Action of Millet Diastase on Starch of Glutinous Variety.

As was previously shown by the writer in this journal,* starches may be classed as common and glutinous varieties, differentiated chiefly by their iodine reactions, the former being coloured blue and the latter red, and the conversion product of starches of the glutinous variety by malt diastase contains a greater amount of dextrin and less of maltose than in the case of starches of the common variety.

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* Y. Tanaka, this Journal, Vol. IV. No. 2, 15—29.

Now, the starch of glutinous millet which is one of the glutinous variety, was hydrolysed by millet diastase. 1000 c.c. of a paste containing about 3 per cent. of glutinous millet starch were hydrolysed with the precipitated millet diastase from 25 grms. of glutinous millet at 55°C. for 5 hours. The paste was liquefied in 15 minutes and the final result was as follows:

Time of hydrolysis in hours.	Grms. of Maltose in 100 c.c. of saccharified solution.	Iodine reaction.
1	—	Red
2	—	Red
3	0·28	Red

Comparing the result with that in the case of potato starch it may be seen that the hydrolytic product of starch paste of this kind by millet diastase contains a much smaller quantity of maltose, but the liquefaction of the paste occurs more rapidly.

The Combined Action of Millet and Barley Diastases.

From the foregoing investigations, it may be seen that the diastase contained in glutinous millet is not identical with the diastase in ungerminated barley which slowly liquefies starch paste and rapidly saccharifies the same; that is, millet diastase exerts a rapid liquefying action and a slow saccharifying action on starch paste. This is rendered more obvious when millet diastase is mixed with barley diastase and their combined action which is very vigorous, is ascertained. Two experiments were made with the following results.

(a) About 4 grms. of potato starch were gelatinised with 150 c.c. of water and the hydrolysis with the precipitated diastase was carried out at 55°C. for one hour, and tested:—

Diastase added.	Maltose produced.	Time of liquefaction.
(i) Diastase from 5 grms. of barley	2·59 grms.	3 hours
(ii) Diastase from 5 grms. of glutinous millet	0·41 „	30 minutes
(iii) Diastase from 2·5 grms. of barley + Diastase from 2·5 grms. of glutinous millet	3·01 „	40 minutes

(b) About 4 grms. of glutinous millet starch were gelatinised with 100 c.c. of water and the hydrolysis with the precipitated diastase was carried out at 55 C. for 5 hours, and tested:—

Diastase added.	Maltose produced.	Time of liquefaction.
(i) Diastase from 5 grms. of barley	2·50 grms.	3 hours
(ii) Diastase from 5 grms. of glutinous millet	0·62 „	15 minutes
(iii) Diastase from 2·5 grms. of barley + Diastase from 2·5 grms. of glutinous millet	2·74 „	20 minutes

Summary.

1. The optimum temperature for hydrolysis with millet diastase is higher than that of the barley diastase, being about 50°—55° C.

2. The hydrolysing action of millet diastase differs from those of barley and other cereals: it liquefies the starch paste to soluble starch very rapidly, and hydrolyses the latter successively to dextrins with the simultaneous formation of a comparatively small amount of maltose until no other dextrin than achroodextrin remains. Since the diastase has a strong liquefying but

weak saccharifying power, it acts on starch paste and soluble starch yielding equal amounts of maltose at equal intervals.

3. The final product of the hydrolysis of starch paste or soluble starch by millet diastase consists only of maltose and achroodextrin; erythrodextrin and *α*-erythrodextrin are produced in the intermediate stages. *α*-erythrodextrin is probably more complex than erythrodextrin, and is coloured red by iodine and very slowly saccharified by millet diastase.

4. The millet diastase liquefies starch paste of the glutinous variety more rapidly but saccharifies it more slowly than the common starch paste.

5. The combined action of millet and barley diastases on starch paste is more vigorous and produces a much greater amount of maltose than that of either diastase alone.

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Nitro-colouring Matters derived from Fukugiflavin.

By

E. Itō, *Kōgakushi.*

The writer lately prepared new nitro-colouring matters from fukugiflavin.* Nitrating it direct with fuming nitric acid, concentrated nitric acid, or with a mixture of sulphuric and nitric acids has not given a good result; while converting it first into its sulphonic acid and then treating the latter with nitric acid has proved satisfactory.

Hence the writer has made experiments by changing severally the quantities of both the acids and also the method of procedure, and has obtained products of a different nature. Among them the following two, for which he proposes the names *Nitrofukugiflavin* and *Nitrofukugiflavin G* respectively, are especially noteworthy as useful nitro-colouring matters.

Nitrofukugiflavin.

10 parts of fukugiflavin are gradually introduced into 200 parts (by weight) of sulphuric acid by stirring constantly. The whole is warmed on a water-bath until the insoluble portion is no longer recognizable, and a sample taken out dissolves completely in water. The solution thus obtained, which is deep violet in colour

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* E. Itō, this Jour., Vol. II. No. 5, 267-279.

and viscid in consistency, is kept cold by means of a freezing-mixture. 130 parts of concentrated nitric acid are then slowly poured into the above solution, stirring constantly and keeping the temperature of the solution below 10°C. The stirring is continued for about one hour after the last portion of nitric acid has been added.

The product is then poured into a cold water, when the resulting nitro-compound at once separates out. It is collected on a filter and washed with a small quantity of cold water. The product is then dissolved in water, and common salt is added in order to salt out the nitro-colouring matter, which thus readily appears as a yellow precipitate. The precipitate is collected on a filter and washed with salt-water, and finally dried at ordinary temperatures.

This nitro-colouring matter, for which the writer proposes the name *Nitrofukugiflavin*, is an orange-yellow powder, having a bitter taste and an explosive character. It is perfectly soluble in water, and the aqueous solution dyes animal fibres, direct, a bright yellow with a somewhat orange tinge. Its tinctorial power and equalising property are remarkable. It can be used both *per se* and in the production of mixed colours.

Nitrofukugiflavin G.

10 parts of fukugiflavin are dissolved in 120 parts of sulphuric acid in the same manner as in the case of nitrofukugiflavin. The solution is then slowly poured into 100 parts of concentrated nitric acid, while being well stirred. The temperature must be kept at 80—100°C. during this reaction.

The whole is then poured into a large bulk of cold water and the acids are nearly neutralized with calcium carbonate. The liquid part is decanted off and the nitro-colouring matter is then salted out with common salt. The precipitate is separated from the liquid part and finally dried at ordinary temperatures.

The colouring matter thus obtained, for which the writer proposes the name *Nitrofukugiflavin G*, is quite similar to Nitrofukugiflavin in all properties, except that the yellow produced with the former is more greenish than that produced with the latter; it, therefore, need not be described in detail.

Conclusion

From the results of the present research, the writer is led to the following conclusion:—

1. By nitrating[*] the fukugiflavin nitro-colouring matters can be produced.

2. As to the method of nitration, the so-called direct method does not give good results, while converting first into the sulphonic acid and then nitrating answers satisfactorily.

3. The writer has thus prepared two nitro-colouring matters, for which he proposes the names, *Nitrofukugiflavin* and *Nitrofukugiflavin G* respectively. The former dyes animal fibres direct a handsome yellow and the latter a yellow with a greenish tinge, and both of them display great tinctorial power and a high equalizing property.

4. Thus the insoluble natural mordant dye, fukugiflavin, can be converted into soluble subjective dyes, which will find wider application than the original.

June, 1907.

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[*] Such treatment may also be applied to other natural colouring-matters than fukugiflavin, if they are prepared in such a convenient form as fukugiflavin. New ways of application may thus be found for other natural colouring-matters.

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On the Japanese Dyewood "Doss"

By

E. Itō, *Kōgakushi.*

Doss, *Ilex Mertensii, Maxim.*, is an ever green tree found only in the Ogasawara and Okinawa (Loo Choo) islands of Japan. It grows largely in the latter, whence the writer has secured samples of its bark and extract.

It contains some tannin substance and is often used experimentally in dyeing as a substitute for myrica-bark (*Myrica rubra, Sieb. et Zucc.*), which is now becoming scanty in the Okinawa district. But such experimental applications have all ended in unsatisfactory results.

The writer has also taken up the subject and made some experiments with this bark. But the results hitherto obtained have also been unsatisfactory. During the present research, however, he has been led to the discovery of a new yellow colouring-matter existing in this bark. Hence he has turned his research in this direction, and the results thus far obtained are set forth in this paper.

In consequence of the present research, it has now become evident that the doss-bark or its extract can not be used as a substitute for the myrica-bark, unless some new mode of application is devised, since it contains too much yellow colouring-matter to be employed in the same manner as the myrica-bark.

Experimental.

The coarsely powdered doss-extract was dissolved in five times its weight of water, and the solution was evaporated to a small bulk under a reduced pressure, and poured into a large quantity of alcohol, when a dark brown viscous precipitate separated out, and was filtered off. The filtrate was again evaporated down to a viscid consistency and was extracted with boiling alcohol. On allowing the alcoholic extract to stand, the impure colouring matter separated out as a handsome yellow precipitate, which was collected with the filter-pump, and allowed to dry in a vacuum-dessicator.

For further purification, the product was dissolved in dilute alcohol, alcoholic lead acetate solution added, the resulting yellow precipitate removed, the filtrate evaporated to a small bulk and poured into a large bulk of ether, which caused the separation of a resinous impurity. The pale yellow, ethereal liquid was well washed with water, evaporated to dryness, and the residue was treated with boiling alcohol, the alcoholic solution being left to stand for a few days. Minute crystals slowly separated, which were collected, washed with a small amount of alcohol, and crystallised first from dilute ethyl alcohol and then from methyl alcohol until the melting-point was constant.

The product thus purified consisted of light yellow needles, which melted at 271–272°C. The results of analyses are as follows:—

	Found		Calculated for
	(i)	(ii)	$C_{15}H_9O_5$
C	66·75	66·83	66·91
H	3·41	3·33	3·35 per cent.

This new colouring-matter, for which the name *dossetin* is proposed, is little soluble in hot alcohol, benzol, and ether. It dissolves in cold sulphuric acid with a dull yellow colouration, and the solution, on being heated, becomes a dull violet-red, and diluting with water produces no precipitate. With alum, stannous chloride and lead acetate, yellow precipitates are produced, while ferric chloride developes a brownish yellow colouration. Alcoholic potassium acetate gives no insoluble salt, and mineral acids do not react to form compounds.

The fresh decoction of doss-bark has a reddish brown colour, but soon becomes turbid. This solution is slightly acidic and gives the following reactions:—

Alkalies: the solution turns yellow.

Dilute acids: a turbidity is produced, which redissolves on boiling, and the solution becomes slightly reddish on cooling.

Alum: } pale while precipitate.
Basic alum: }

Stannous chloride: ditto.

Lead acetate: dirty orange precipitate.

Ferrous sulphate } : dirty olive precipitate, which, on boiling, changes to brown.
Ferric chloride }

Copper acetate: pale white precipitate.

Doss dyes mordanted fabrics in the manner of polygenetic dyes. The following table shows the hues produced on fibres with the different mordants.

Mordant	Cotton	Wool	Silk
Alumina	Dull yellow	Dull yellow	Dull yellow
Tin	Yellow	—	Bright yellow
Chrome	Light olive	Orange yellow	Golden yellow
Iron	—	—	Olive

As to methods of application, the writer has preferably adopted the following:—

Silk with alumina mordant.—The silk is put into a basic alum solution containing 60 grms. of alum and 6 grms. of soda crystals per litre, worked about for a quarter of an hour and left to soak over night. It is then evenly wrung and put in a solution of silicate of soda (0·5° Tw.) for a quater of an hour, and then carefully washed. The silk thus mordanted is put into a dye-bath containing the solution of doss-extract, the bath being then slowly raised to the boiling-point in about one hour, and kept thereat for twenty minutes more, wrung out, washed well, and finally dried.

Silk with chrome mordant.—The silk is steeped in a solution of chromium chloride (16° Tw.) and left to soak over night. It is then evenly wrung, washed, passed through a solution of silicate of soda (1° Tw.) for half an hour, and washed well. Then dyed with doss-extract in the same manner as above stated.

Silk with tin mordant.—The silk is steeped in a solution of stannous chloride (6° Tw.) and left to soak over night, and then passed through a dilute solution of sodium carbonate and washed. It is then dyed as above.

Silk with iron mordant.—The silk is steeped in a solution of pyrolignite of iron (13° Tw.) for one hour, passed through a dilute solution of sodium carbonate, and washed. It is then dyed as above.

Wool with alumina mordant.—The wool is put into a mordanting bath containing 10% (of its weight) of alum, 3% of oxalic acid and 1·5% of tartar, the bath being slowly raised to the boiling-point for about one hour, kept thereat for half an hour more, and thoroughly washed. It is then dyed as above.

Wool with chrome mordant.—The mordanting bath is prepared

with 3% of potassium bichromate and 2·5%of tartar. The wool is mordanted in this bath in the same manner as the previous case. It is then dyed as above.

Cotton with alumina mordant.—The cotton is first impregnated with Turkey-red oil (dissolved in 10 times of water), wrung evenly and dried. It is then steeped in a basic alum solution (5° Tw.), left to soak over night, passed through a chalk-bath heated to the temperature of 45° C., and finally washed well. It is then dyed as above.

Cotton with chrome mordant.—The cotton oiled as above is steeped over night in a tannic acid solution (3 %), and washed. It is then steeped over night in a chromium chloride solution (15° Tw.) and passed through a dilute solution of sodium carbonate. It is then dyed as above.

Cotton with tin mordant.—The cotton oiled as above is steeped over-night in a tannic acid solution (3 %) and washed. It is then steeped one night in a stannous chloride solution (6° Tw.) and thoroughly washed. It is then dyed as above.

The tinctorial power of this dyestuff is not so great, and the colours produced are not so bright as in the case of fukugi and other ordinary yellow dyes. So the writer is of opinion that this dyestuff, though being of some interest to science, will not be a valuable addition to the colouring matters already in use, unless some new way of application is devised.

Summary.

1. Doss* (*Ilex Mertensii, Maxim.*) contains a new colouring-

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* Plants belonging to the genus *Ilex* are usually considered to contain no colouring matter; but the present result shows that the above opinion does not hold true for all the plants belonging to this genus.

matter called *dossetin* $C_{15}H_{8}O_{5}$, crystallising in yellow needles and melting at 271–272° C.

2. Doss-extract may be employed as a mordant dyestuff of a polygenetic nature. Thus, in general, the following hues are produced: with alumina mordant, dull yellow; with tin, a beautiful yellow; with chrome, orange-yellow; and with iron, olive.

3. Its tinctorial power is not so great, and the dyed-colours are not, in general, so bright as to warrant its being regarded as a valuable addition to the hitherto existing yellow colouring matters, unless some new method of application be found.

The writer desires to acknowledge his indebtedness and to express his thanks to Mr. K. Shibata, *Rigakuhakushi*, for valuable suggestions to the botanical nature of the present plant, based on his elaborate examination.

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On the Composition of Chrysalis Oil.

By

Mitsumaru Tsujimoto, *Kōgakushi.*

Silk being the principal industrial product of Japan, not an inconsiderable quantity of chrysalis waste is annually produced in the manufacture of it.

Formerly the chrysalis was chiefly used as a fertiliser or as food material for fish. Lately, however, attention has been drawn to the use of the oil contained in it, with the result that now generally throughout the silk-producing districts of Nagano and other prefectures the oil has become a promising by-product.

The chrysalis oil is usually obtained in this country by steaming the powdered dry chrysalis and then pressing it in a screw or wedge press. The very dark coloured and ill-smelling oil thus obtained is, after some purification, used as a material for soap-making; while the press cake is valued highly as a fertiliser, as it is very rich in nitrogenous substances.

As this chrysalis oil is the first oil obtained from the bodies of worms, a close study of its nature is not only necessary for its industrial application, but is also interesting from a scientific point of view.

The literature on the subject is, however, still very scanty,[1]

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[1] The reports on chrysalis oil published by the writer in Japanese journals are as follows:
"A report on chrysalis oil manufacture in Nagano and Gunma prefectures." (Report of Industrial Experiment Station, Tokyo. Vol. II. 1905, 473.)
"On chrysalis oil." (Kōgyō Kwagaku Zasshi. 1905, 365.)
"On the fatty acids of chrysalis oil." (Ibid. 1905, 835)

and it is only quite recently that the oil has attracted the notice of foreigners. Dr. J. Lewkowitsch[1] has published two papers on the properties and unsaponifiable matter of the oil. His investigations are doubtless the first reports on chrysalis oil published in foreign languages.

EXPERIMENTAL.

Dried chrysalis waste from spring silkworms was procured from the filature of Mr. Kozaemon Ito, the well known silk manufacturer of Muroyama, in Miyé prefecture. One litre of the chrysalis weighed 293 grams. On analysis, it was found to contain moisture 5·48%, oil 26·26% and ash 3·77%.

The chrysalis was dried in a steam oven for a few hours and powdered in a mortar. It was then exhausted in an extractor with petroleum ether with a boiling point below 80° C. After exhaustion, the ether was distilled off from the extracted oil, the last trace of it being removed by passing a current of dry carbon dioxide through the oil heated to 110° C.

The chrysalis oil thus obtained is an yellowish red liquid with a slightly unpleasant odour; on cooling, it solidifies at 0° C. The following values were determined.

Specific gravity (15·5° C)	0·9280
Acid value	18·68
Saponification value	194·12
Iodine value (Wijs)	131·96
Hehner value	94·5
Reichert Meissl value	3·38

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[1] Ztschr. f. Unters. d. Nahr. u. Genussmittel. Bd. XII, Heft 11. (Journ. Soc. Chem. Ind., Abstr. 1907, 24).

Ibid., Bd. XIII, Heft 9. (Ibid., Abstr. 1907, 622).

True acetyl value (Lewkowitsch)	19·72
Refractive index (20° C)	1·4757

In the following experiments, this oil was exclusively used.

I. UNSAPONIFIABLE MATTER.

To determine quantitatively the unsaponifiable matter present in the oil, about 10 grams of the sample was heated with 50 c. c. of 8% alcoholic soda solution. After saponification, the paste was dissolved by adding a fresh quantity of alcohol; then 5 grams of sodium bicarbonate and about 50 grams of previously ignited sand were thoroughly incorporated into it. The mass was dried, powdered and extracted in a Soxhlet extractor with light petroleum spirit.

The extracted substance was again boiled with a small quantity of alcoholic potash solution in order to ensure complete saponification; the alcohol was then evaporated off, the residue dissolved in petroleum ether, and transferred to a separating funnel.

The ether layer, after several washings with water, was finally drawn off into a tared flask. By distilling off the solvent, the remaining unsaponifiable matter was dried and weighed.

As the result of two analyses, an average of 1·63% was obtained with this sample of the oil.

To obtain some quantity of the unsaponifiable matter for further examinations, 200 grams of the oil were treated in the same manner.

The unsaponifiable matter is a white crystalline solid. It does not melt at 100° C, and gives the characteristic colour reaction of cholesterol (or phytosterol), when concentrated sulphuric acid is added to its chloroformic solution.

By recrystalizing it from 90% alcohol, crystals of m. p. 138·5°C were obtained, which after repeated purification, melted at 143·5° C.

This substance crystallizes in long prismatic forms which examined under the microscope closely resembled those of phytosterol.

The substance dried at 100° C gave the following result by elementary analysis.

0·0563 gave 0·1724 CO_2 and 0·0605 H_2O.

C=83·51; H=12·03.

$C_{26}H_{44}O$ requires C=83·79; H=11·91 per cent.

To decide whether this substance was cholesterol or phytosterol, it was acetylated by heating with an excess of acetic anhydride according to Bömer's method. The acetate thus formed melted at 125·5° C.

From these results, it was concluded that the unsaponifiable matter in chrysalis oil consists mainly of phytosterol.[1]

On recrystallizing the unsaponifiable matter from 90% alcohol for the first time, a somewhat large quantity of a wax-like substance (m. p. below 100°C) insoluble in the solvent, was observed. The nature of it has not yet been ascertained.[2]

II. ALCOHOLIC RADICAL.

There is little doubt that chrysalis oil belongs to fatty oils, i. e. consists of glycerides. In the following experiments, I practically isolated glycerol from it and determined its content quantitatively.

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[1] This apparently exceptional result. contradictory also to the result published by Lewkowitsch (*loc. cit.*) led the writer to perform the experiment with another sample of chrysalis oil. An oil from Shiga prefecture was used for the purpose. The results obtained in this case closely agreed with that above-mentioned. The crystal form, m. p. (137–139° C) and the acetate (m. p. 123° C) indicate the presence of phytosterol in the sample.

[2] This substance is probably the same as the hydrocarbon described by Lewkowitsch (*loc. cit*).

Now, inasmuch as chrysalis oil contains a small quantity of soluble fatty acids, if an oxidation method such as the bichromate method be adopted for the determination of its glycerol content, there will be an error originating from the presence of soluble acids in the aqueous filtrate. So I adopted the extraction method devised by A. A. Schukoff and P. J. Schestakoff.[1] The process of determination recommended by them is as follows.

10 grams of the sample are saponified with alcoholic potash solution as usual, the alcohol then evaporated, the soap dissolved in water and decomposed with diluted sulphuric acid. The aqueous filtrate from the insoluble fatty acids being neutralised to a weak alkaline reaction with potassium carbonate, is evaporated down to syrupy consistence at a temperature not higher than 80°C.

About 20 grams of powdered anhydrous sodium sulphate are then mixed with it, and the mass is extracted in a Soxhlet apparatus with acetone for about five hours. From the extract the acetone is distilled off, and the residue is dried at 75–80° C and weighed.

By this process, 9·42% of the extract was obtained from this sample of chrysalis oil.

This substance is a brownish coloured viscous liquid of sweet taste, which is soluble in neither ether nor petroleum spirit, but readily soluble in alcohol.

0·1694 gave 0·2388 CO_2 and 0·1314 H_2O.

C=38;44; H=8;68.

$C_3H_5(OH)_3$ requires C=39·10; H=8·76 per cent.

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[1] Zeits. für angew. Chemie, 1905, 294.

Hence, the substance was identified as glycerol.

III. FATTY ACIDS.

The insoluble fatty acids obtained by the usual method were used for examination. They contain, therefore, some unsaponifiable matter which when necessary, was removed from them.

As the soluble or volatile fatty acids of chrysalis oil are not only small in quantity as might be expected from the comparatively low Reichert-Meissl value of the oil, but also appear to be secondary products of decomposition, the examination of them was wholly omitted in the present experiment.

The insoluble fatty acids of chrysalis oil form a crystalline mass at the ordinary temperature. The constants were determined with the following results:

Specific gravity (at 100°C. Water at 15·5° C=1)	0·8513
Melting point	36·5°C
Solidifying point	27 to 28° C
Neutralisation value	199·34
Mean molecular weight	281·43
Iodine value (Wijs)	135·83

By the lead-salt-ether method, the following result was obtained:

Unsaturated acids (I. value 178·73)	75 %
Saturated acids (M. p. 57° C.)	25 % (by diff.)

(1) *Examination of the Saturated Fatty Acids.*

To test for the presence of stearic acid, about 5 grams of the mixed acids from which the unsaponifiable matter had been removed, were treated by Hehner and Mitchell's method. After having been kept at 0° C over-night, a small deposit of crystals was observed. When separated from the mother liquor and washed with alcohol, it melted at 58° C. It is, therefore, not stearic acid, or if any of the latter be present in it, there must be some other acid or acids higher than palmitic admixed with it.

To perform the fractional precipitations of the acids, 20 grams of raw solid acids (m. p. 42° C.) obtained by treating the lead soap with ether, were dissolved in 200 c. c. of 90% alcohol, and fractionated into ten parts each with 4 c. c. of 20% alcoholic solution of magnesium acetate. At the eighth precipitation, however, no deposit was obtained owing probably to the rather high content of liquid acids in the original sample of the solid acids. The magnesium salts were decomposed with diluted hydrochloric acid and the melting points of the liberated acids were determined, the results being as follows:

Precipitated acids	Melting pts.
1st	55·6° C
2nd	55·8
3rd	59·2
4th	60·0
5th	60·2
6th	60·0
7th	61·0
8th	liquid.

The 4th, 5th and 6th acids which have nearly the same melting point, were united and recrystallized from alcohol. The first crop melted at 60·5°C, the second at 61·5°C and the third at 60·2°C. This second acid on combustion analysis gave the following composition.

0·1010 gave 0·2794 CO_2 and 0·1137 H_2O

$C=75·45$; $H=12·60$

$C_{16}H_{32}O_2$ requires $C=74·93$; $H=12·59$ per cent.

The substance is therefore palmitic acid.

The 1st and 2nd precipitated acids from the original sample were united and fractionated into four parts. The melting point of the first precipitated acid is 56·8°C, the second and third 55·6°C. (the fourth not determined). Further examinations of these acids, such as elementary analysis, determination of neutralisation value, etc. were tried, but none of them led to definite conclusions as to the identification of these compounds. It is, however, very probable that there may be present some fatty acid which is more difficultly soluble in alcohol than palmitic acid, but melts at a lower temperature than the latter.

(2) *Bromination of the Unsaturated Acids.*

The unsaturated fatty acids of chrysalis oil gave an ether-insoluble product on bromination. By the usual treatment, 11·94% of the compound was obtained from the mixed fatty acids.

It was a white powder, very difficulty soluble in ether, alcohol or glacial acetic acid, but soluble in the last solvent when boiled in it. It melts at 178° C to a clear liquid.

0·2403 gave 0·2522 CO_2 and 0·0898 H_2O

0·2304 gave 0·3399 Ag Br.

$C=28·62$; $H=4·18$; $Br=62·78$.

$C_{18}H_{30}O_2Br_6$ requires $C=28·50$; $H=3·99$; $Br=63·29$ per cent.

This substance is therefore linolenic hexabromide; hence, the presence of linolenic acid in the oil was confirmed.

As 100 parts of the hexabromide corresponds to 36·7 parts of linolenic acid, the amount of the latter occurring in the oil should be 4·38% of the mixed fatty acids.

This rather remarkable content of linolenic acid in chrysalis oil is worthy of special mention, since it has until recent times been generally believed that such highly unsaturated acids as linolenic acid were not present in oils of animal origin.

To examine for the existence of tetrabromides, 10 grams of the unsaturated acids were dissolved in 50 c. c. of petroleum ether and brominated; the ether and the excess of bromine were evaporated off on a water bath. The residue again treated with 50 c. c. of hot petroleum ether and filtered (the hexabromide remaining on the filter as an insoluble residue).

On cooling the filtrate to 10° C, no crystal was obtained but only a small quantity of liquid substance; so tetrabromides (at least that derived from linolic acid) appear not to be present in it.

On evaporating off the petroleum ether from the above filtrate, a very viscid oily substance was left. This seems to consist chiefly of dibromides, but owing to its containing impurities, the examination of it was abandoned.

(3) *Oxidation of the Unsaturated Acids.*

30 grams of the unsaturated acids were oxidised according to Hazura's method. After passing sulphur dioxide into the solution, the whole was filtered through filter paper, and thus divided into (a) precipitate and (b) filtrate.

(a) Precipitate.

This was a white solid mass with which a small amount liquid substance was admixed. On drying it weighed 17 grams. It was first washed with a small quantity of ether, and treated with 1½ litres of ether. From the ethereal solution, the ether was distilled off, and the residue crystallized from alcohol. Crystalline laminae having a pearly lustre were deposited. They melted at 131-131·5° C.

0·0753 gave 0·01874 CO_2 and 0·0769 H_2O.

C=67·87; H=11·34.

$C_{18}H_{34}O_2(OH)_2$ requires C=68·29; H=11·47 per cent.

The substance is therefore dihydroxystearic aid derived from the oleic acid present in the original acids.

The portion insoluble in ether was boiled with 1½ litres of water and filtered rapidly. On cooling, the filtrate became turbid, and fine white crystals were deposited, which melted at 196–197°C. This substance is not easily soluble in alcohol, but is soluble in hot water. To purify it further, it was treated with a small quantity of warm alcohol to remove the admixed impurities; the insoluble portion was then dissolved by boiling in a large quantity of water. On cooling the solution deposited pure white crystals which under the microscope appeared as small rhombic plates. It melted at 203° C.

0·1192 gave 0·2452 CO_2 and 0·1004 H_2O.

C=56·10; H=9·43.

$C_{18}H_{30}O_2(OH)_6$ requires C=56·80; H=9·54 per cent.

Hence, this substance is linusic acid; so the presence of linolenic acid in the unsaturated acids was confirmed.

The residue from linusic acid was again boiled with 1½ litre of water. On cooling, fine white crystals of m. p. 154° C were obtained.

0·0718 gave 0·1651 CO_2 and 0·0713 H_2O.

C=62·71; H=11·11.

$C_{18}H_{32}O_2(OH)_4$ requires C=62·02; H=10;42 per cent.

The substance is not yet a single compound, but the result of its elementary analysis closely approaches that of sativic acid. By repeated purifications from alcohol and separations by means of the solubilities of barium salts, several compounds with melting points at 165° C, 174° C etc were obtained. Thus it is very likely that this substance is a mixture of several tetrahydroxy acids derived from linolic or its isomeric acids.

There was still an insoluble residue remaining after the extraction of the above compounds. As it seemed to be a part of the tetrahydroxy acids, the examination of it was omitted.

(b) Filtrate.

The volume of the filtrate amounted to about 4 litres. It was neutralised with caustic potash, evaporated down to about 300 c. c. and acidified with diluted sulphuric acid. The hydroxy acids and inorganic salts thus precipitated were drained on a porous tile; and treated with ether to remove the secondary products of oxydation; then the hydroxy acids were extracted with absolute alcohol. The portion insoluble in alcohol was recognized to be inorganic salts consisting chiefly of $MnSO_4$ and K_2SO_4; so it was thrown away.

The alcoholic solution, on concentration, deposited white crystals. They were dissolved in water and recrystallized. The portion first deposited being removed, the second crop was taken out and examined. It melted at 174° C. By recrystallizing it from water, the melting point became 171° C. It is readily soluble in warm water.

$$0{\cdot}1157 \text{ gave } 0{\cdot}2382\ CO_2 \text{ and } 0{\cdot}0989\ H_2O.$$

$$C=56{\cdot}15;\ \ H=9{\cdot}57.$$

$$C_{18}H_{30}O_2(OH)_6 \text{ requires } C=56{\cdot}80;\ H=9{\cdot}54 \text{ per cent.}$$

The substance is therefore isolinusic acid derived from isolinolenic acid.

No other important compound was found in the filtrate.

IV. SUMMARY.

The results of the present experiments may be summarised as follows:

(1) Chrysalis oil contains 1·63% of unsaponifiable matter which mainly consists of phytosterol.

(2) The alcohol obtained by saponifying the oil is glycerol. With the sample under examination, 9·42% of glycerol was obtained by the extraction method.

(3) The insoluble fatty acids consist of 25% saturated (m. p. 57° C) and 75% unsaturated acids (iodine value 178·73. Unsaponifiable matter not separated).

(4) Amongst the saturated acids, palmitic acid was identified. Stearic acid is probably not present.

(5) The unsaturated acids consist of oleic, linolenic and isolinolenic acids. Besides them, some isomers of linolic acid are also present. By calculating from the ether-insoluble hexabromide, the amount of linolenic acid occurring in the oil appears to be 4·38% of the mixed fatty acids.

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On a Few Japanese Vegetable Oils.

By

Mitsumaru Tsujimoto, *Kōgakushi.*

Several vegetable oils and fats are obtained in Japan from the seeds of plants that are indigenous to the country. Some of these are produced on a commercial scale and are already exported to Europe and America, where their properties and applications have been scientifically investigated and determined; but there are many others that are still scarcely known to foreigners. Even in this country, the chemical properties of these oils have as yet been very little studied.

In his "Industries of Japan" (pages 150 to 160), Dr. J. J. Rein gives an account of oil manufactures in Japan, including brief descriptions of *tsubaki*, *sasanqua*, *kaya*, and *inukaya* oils. His statements on the properties of these oils, however, are incomplete and sometimes erroneous.

In the following pages, I shall describe a few Japanese vegetable oils which I have examined.

Tsubaki Oil.

This oil is obtained from the seeds of the tsubaki tree, *Thea japonica Nois.* (*Camellia japonica L.*), a plant of the family, *Theaceae.* The tree is generally planted in gardens for ornamental purposes on account of its beautiful flowers; but in certain districts of the country, it grows wild in some abundunce, so that the collected seeds are locally worked out for the preparation of the oil. The places noted as oil-producing districts are the Islands of Idzu (Ōshima, Niijima, Miyakejima, Hachijyōjima etc), Shidzuoka prefecture, and several prefectures of Kiushiu (among others Kumamoto, Nagasaki and Ōita).

The tsubaki tree grows to a height of from ten to twenty feet; blossoms in early spring; and in autumn the fruit ripens and the seeds contained in it fall to the ground. In the Idzu Islands, the gathering of the seed is chiefly done by the women.

The seed is of a somewhat hemispherical form with a hard lustrous dark brown testa; it is from 2 to 2·5 centimeters in length, and in weight varies from 1·2 to 3 grams. The nucleus is pale yellow and is rich in oil. A specimen from Ōshima examined by me consisted of 41·5% testa and 58·5% nucleus. The analyses of nuclei from different localities gave the following results:

Locality	Moisture	Oil	Ash
Ōshima	4·93%	64·36%	1·75%
Miyakejima	5·58 ,,	64·33 ,,	1·60 ,,
Tokyo	4·24 ,,	66·42 ,,	1·73 ,,

For the preparation of the oil, the seeds are first dried by exposure to sunlight, then crushed, steamed and pressed in the special wooden wedge press, called *tatsugi*, usually employed by Japanese oil manufacturers. The pressing is generally twice repeated. Sometimes the seeds are decorticated previous to crushing. In the Islands of Idzu, this latter process is known as *mukimi-shibori* (decorticated seed pressing); while the former called *karami-shibori* (shell and kernel pressing). Owing to the inequality in the sizes of the seeds, no appropriate apparatus for decortication has yet been devised; so the *mukimi-shibori* process necessarily involves somewhat tedious hand-labour for the separation of the nucleus. No practical difference in the properties of the oils obtained by these processes is, however, recognisable.

By the *karami-shibori* process, about 15% of the oil by volume is obtained from the seed.

Commercial tsubaki oil is usually a yellow coloured liquid with a slight peculiar odour; the inferior grade, however, varies from yellow to reddish yellow, and has a decidedly rancid smell. The oil prepared from fresh seeds by cold pressing has a very pale yellow colour and is practically odourless. The taste of the fresh oil is mild and pleasant.

Several oils from different sources were examined, the results being tabulated as follows:

Physical and Chemical Constants of Tsubaki Oil.

Locality	Specific gravity at 15·5°C	Acid value	Saponification value.	Iodine value (Wijs)	Refractive index at 20°C.	Hehner value	Reichert Meissl value
1. Ōshima (Cold drawn)	0·9161	2·48	191·00	81·32	1·4686	95·6	0·53
2. ,, (,, ,,)	0·9163	1·63	191·48	81·28	1·4688	——	——
3. ,, (karami-shibori)	0·9159	3·32	191·03	80·31	1·4686	——	——
4. ,, (mukimi-shibori)	0·9160	2·29	191·05	80·43	1·4687	——	——
5. Miyakejima (Cold drawn)	0·9162	2·16	192·58	80·31	1·4682	95·3	0·48
6. ,, (,, ,,)	0·9163	2·04	192·53	80·30	1·4683	——	——
7. ,, (commercial oil)	0·9162	8·84	192·57	80·62	1·4679	——	——
8. ,, (,, ,,)	0.9161	4·64	191·39	80.64	1·4691	——	——
9. Niijima (,, ,,)	0·9162	4·62	190·22	80·21	1·4686	——	——
10. Hachijyojima(,, ,,)	0·9166	7·65	191·18	80·07	1·4685	——	——
11. Nagasaki (,, ,,)	0·9164	3·12	189·89	80·82	1·4691	——	——
12. Tokyo (cold drawn)	0·9159	2·67	190·58	80·95	1·4691	——	——

N.B. Oils Nos. (1), (2), (5), (6), (12) were obtained from the respective seeds in the laboratory. Although the acid values belong to the variable, they are mentioned in the table for convenience.

When kept at -10°C for one hour, tsubaki oil is partially solidified; at -15°C, it solidifies completely.

In the common solvents for fatty oils, the oil dissolves readily; in Valenta's test, a sample from Ōshima gave turbidity at 81°C.

By the elaidine test, the oil solidifies in two hours, and after twenty-four hours, a very hard mass not readily penetrable with a glass rod is obtained.

The mixed fatty acids from tsubaki oil consist of solid and liquid parts at the ordinary temperature; in summer, they form a perfect liquid.

The chief constants of the mixed acids from the two samples of the oil previously mentioned are as follows:

Fatty acids from	sp. gr. $\left(\frac{100^{\circ}C}{15^{\circ}C}\right)$	Melt. pt.	Neutralisation value	Mean Mol. weight	Iodine value (Wijs)
Oil No. 1. (Ōshima)	0·8419	22°C	196·75	285·12	83·28
Oil No. 5. (Miyakejima)	0·8402	21·5°C	198·22	283·01	84·15

By the lead-salt-ether method, 93;1% of liquid acids (iodine value 89;86) were obtained from the mixed fatty acids of oil No. 5. (Miyakejima).

Neither the oil nor its acids gave ether-insoluble compounds on bromination.

On oxidizing the mixed acids from tsubaki oil according to Hazura's process, a very large quantity of dihydroxystearic acid was obtained.

The composition of tsubaki oil, so far as at present known, consists essentially of olein. A. Misumi[1] who first published results in this direction, concluded that the solid acids contain palmitic acid, stearic acid being also probably present. The

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[1] Kōgyō Kwagaku Zasshi, 1906, 1 and 111.

liquid acid was found to consist of oleic acid; although a trace of linolic acid was suspected. T. Kametaka[1] was, however, unable to detect sativic acid in the oxidation products of the mixed acids by Hazura's process.

Tsubaki oil is a type of non-drying oil, and closely resembles olive oil in its properties. Its iodine value is even lower than that of the latter. The only marked difference between the two oils is the rather high solidifying point of olive oil, inasmuch as tsubaki oil does not solidify even far below 0°C.

Tsubaki oil, being the highest priced of all Japanese vegetable oils, is grossly adulterated with cheaper oils. The usual adulterants are rape, cottonseed, bean and arachis oils. Besides the systematic examination of the constants, etc., the writer found Bieber's reagent (a mixture of equal parts by weight of water, fuming nitric and concentrated sulphuric acids) very efficient for the detection of other oils. The manipulation I have adopted is as follows.

2 c.c. of the oil to be examined are placed in a test tube, and 7c.c. of the reagent carefully added. At first the colour of the contact zone of the two layers is observed, and then that of the oil after it has been thoroughly shaken; finally, after the lapse of several hours, the change of the colour of the oil is noticed.

Pure tsubaki oil is indicated by a green colour on the zone, and when shaken, a beautiful bluish green colour is developed, which on standing gradually passes to yellowish, and finally to a greenish yellow which lasts for many hours.

In the case of adulterated oils, the green colouration is not so beautiful, and on standing, a more or less pronounced reddish tinge is observed.

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[1] Journ. Tokyo Chem. Soc., 1906, 385.

The following table contains a few observations made by this test.

Adulterant	Colour of Contact zone	Colour of the oil immediately after shaking	Colour of the oil on standing 24 hrs. after shaking
Rape oil 5%	green	green	faintly reddish yellow
„ 10%	green and pale red	reddish yellow	reddish yellow
Arachis oil 5%	green	green	faintly reddish yellow
„ 10%	green and pale red	green	reddish yellow
Cottonseed oil 5%	green with yellow spots	green	orange red
Pure tsubaki oil	green	bluish green	greenish yellow

The reagent should be prepared afresh before testing, and a blank test with pure tsubaki oil placed side by side is also recommended.

Tsubaki oil is highly valued as a hair oil for which purpose it is chiefly used. In the Islands of Idzu, it is consumed as an edible oil. Owing to its non-drying property and low solidifying point, it is sometimes used as a lubricating oil for delicate machinery, such as watchs, etc. The limited production and high price hinders its general use for industrial purposes.

Sasanqua Oil.

This oil is prepared from the seeds of *Thea sasanqua Nois.* (*Camellia sasanqua Thunb*),[1] a Theaceae plant very similar to tsubaki. This tree is planted in gardens like the tsubaki, and there are many varieties of it. It grows wild in several localities in Kiushiu; in the prefecture of Kumamoto, the trees are found on the lower part of Mt. Kinbō and in the Amakusa Islands, where the seeds are collected for the manufacture of the oil.

The seeds of sasanqua resemble in many respects those of the tsubaki, but are smaller. One seed weighs about 0·5 gram, its length varying from 1·0 to 1·5 centimeters. The testa is a dark brown like that of the tsubaki, but not so hard as the latter. The nucleus is light yellow and is covered with a brown coloured tegmen. A sample from Amakusa consisted of 28% testa and 72% nucleus. The analysis of the seed (nucleus) gave the following result:

Locality	Moisture	Oil	Ash
Tokyo	5·78%	59·39%	1·69%
Amakusa	5·11 „	58·10 „	1·78 „

The oil is prepared in Kumamoto by first drying the seed, then powdering, steaming, and finally pressing it in a wooden wedge press. A second pressing is generally performed. About 16 to 17% of oil by volume is obtained by this process.

For the examination of the properties of the oil, an oil obtained from the Amakusa seeds by cold pressing, and another extracted with ether from the Tokyo seeds were used.

Both oils are of pale yellow colours and closely resemble tsubaki oil in general appearance. The values were determined with the following results:

	Amakusa oil	Tokyo oil
Specific gravity (at 15°C)	0·9163	0·9188
Acid value	6·78	0.36
Saponification value	193·90	193·36
Iodine value (Wijs)	82·31	81·67

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[1] A plant named *Camellia oleifera* grown in China appears to be of the same species as sasanqua.

	Amakusa oil	Tokyo oil
Refractive index (at 20°C)	1·4691	1·4961
Hehner value	96·35	——
Reichert-Meissl value	1·17	——

A few tests performed with the Amakusa oil were as follows.

By cooling the oil in a test tube to −5°C. for one hour, a little turbidity was observed; it solidified completely at −9°C.

By the elaidine test, it solidified in two hours into a very hard mass.

With Bieber's reagent, a yellowish green colour was developed, which after twenty four hours, turned to orange yellow (which distinguishes it from tsubaki oil).

The following constants were determined with the mixed acids from the Amakusa oil:

Specific gravity (at 100°C. water at 15·5 = 1)	0·8405
Melting point	28°C
Neutralisation value	199·64
Mean molecular weight	281·00
Iodine value (Wijs)	86·09

By the lead-salt-ether method, 89·93% of unsaturated acids (iodine value 92·90) was obtained from the mixed acids.

By bromination, no insoluble compound in ether was formed.

By the oxidation according to Hazura's method, a large quantity of dihydroxystearic acid was obtained; therefore the chief constituent of sasanqua oil was confirmed to be olein.

Thus, sasanqua oil resembles tsubaki oil in many of its properties; but as its solidifying point and iodine value are a little higher than those of the latter, it may be presumed that the oil contains more solid and higher unsaturated acids.

Sasanqua oil is chiefly used for hair oil as a substitute for tsubaki oil, and sometimes it is used for lubricating purposes.

Kaya Oil.

This oil is obtained from the seeds of *Torreya nucifera S. et Z.* (*Taxaceae*). The plant grows wild in mountaineous regions in several districts of Japan, notably in Shiga, Osaka, Wakayama, Aichi, Aomori, and the Sanindō prefectures.

The kaya seed is usually of a long oval shape with pointed ends. It is 1·5 to 2·5 centimeters long, and weighs on an average 1 gram.

The testa has a brick brown colour and is very hard; the nucleus is pale yellow covered with reddish brown tegmen. A sample consisted of 32% testa and 68% nucleus.

The analysis of the nucleus gave the following results:

Locality	Moisture	Oil	Ash
Tokyo	5·04%	51·07%	2·20%
Shiga	2·67 ,,	51·70 ,,	2·29 ,,

The oil is prepared by steaming and pressing the powdered seeds. About 13%by volume of the oil is obtained from the seed.

For the examination of the oil, a cold drawn oil from the Tokyo seeds, and two commercial specimens from Miyé and Tottori prefectures were used. The oil obtained by cold pressing is a light yellow liquid with a faint odour and mild taste. When cooled down even to −20°C., it gave no turbidity. The commercial oils are yellow in colour and have faint resinous odour. Their taste is decidely unpleasant. The Tottori oil was very turbid from the presence of admixed impurities.

	Tokyo oil	Miyĕ oil	Tottori oil
Specific gravity (at 15°C)	0·9238	0·9233	0·9244
Acid value	1·48	4·24	12·66
Saponification value	188·38	188·34	187·95
Iodine value (Wijs)	142·21	137·95	133·37
Hebner value	95·7	——	——
Reichert Meissl value	0·93	——	——
Refractive index (at 20°C)	1·4770	1·4760	1·4757

Kaya oil dissolves readily in the common solvents; in Valenta's test, the cold drawn oil gave turbidity at 62°C.

By the elaidine test (at 15°C), no solidification was observed after two hours, and at the end of twenty-four hours, only a small deposit of a buttery substance was formed.

The mixed fatty acids obtained from the cold drawn oil form a pale yellow liquid at the ordinary temperature; when cooled, crystalline deposits were formed, which melted into the oily part at 25°C. The following constants were determined:

Specific gravity (at 98°C)	0·8509
Neutralisation value	192·81
Mean molecular weight	290·96
Iodine value (Hübl)	149·45

When heated in a thin layer at 100°C. for three hours, kaya oil dries to an elastic film. The boiled oil prepared by heating the oil with manganese borate or resinate dries perfectly in twenty-four hours at the ordinary temperature.

By the hexabromide test, no precipitate was obtained.

The composition of kaya oil has not yet been fully ascertained. T. Kametaka[1] obtained a tetrabromide of m.p. 114°C by brominating the mixed acids in glacial acetic acid; so the presence of linolic acid should be taken as proven.

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[1] Journ. Tokyo Chem. Soc., 1906, 387.

Kaya oil belongs to the group of drying oils. Its drying property is, however, much weaker than that of linseed oil, as might be expected from its iodine value.

The cold drawn oil is used as an edible oil; commercial oils have sometimes a very unpleasant resinous odour, and are utterly unfit for eating. Besides, the oil is used for burning, in the manufacture of oil papers, as an insecticide, etc. It may be also used as a material in the manufacture of paint and varnish.

Inukaya Oil.

This oil, called *bĕbĕ* oil in Shiga and Fukui districts, is obtained from the seeds of inukaya, *Cephalotaxus drupacea S. et Z.*, an evergreen plant belonging to the same family (*Taxaceae*) as the kaya tree. The oil is chiefly manufactured in the northern parts of Shiga prefecture.

The inukaya seed is of a small ellipsoidal shape having a brown coloured testa; the nucleus is light brown and possesses a resinous odour. One seed weighs usually 0·5 gram, consisting of 33% testa and 67% nucleus.

The analysis of the seed (nucleus) from Shiga prefecture gave the following result:

Moisture	4·57%
Oil	67·10 „
Ash	2·20 „

The oil is prepared in the same way as kaya oil.

A cold drawn oil was prepared in the laboratory from the above seed for examinations.

This oil is a light yellow liquid of a slightly resinous odour. Cooled to -15°C, no solidification was observed.

Specific gravity (at 15·5°C)	0·9250
Saponification value	188·54
Iodine value (Wijs)	130·33
Refractive index (at 20°C)	1·4760

Heated in a thin layer at 100°C., drying was effected after four hours.

In many of its properties, inukaya oil resembles kaya oil, though it dries less than the latter.

The oil may be used for the same purposes as kaya oil excepting the edible uses, for which its resinous odour seems to render it unacceptable.

Kusu Oil.

This oil is contained in the fruit of the camphor-yielding tree kusu, *Cinnamomum Camphora Nees.* (*Lauraceae*). A sample of the fruit was procured from Fukuoka prefecture. It is grey in colour of a small spherical shape, in diameter about 0·5 centimeter, and weighs on an average 0·1 gram. The analysis gave the following result:

Moisture	6·78%
Oil	42·37 ,,
Ash	2·05 ,,

The oil was prepared in the laboratory by powdering and pressing the fruits at somewhat elevated temperatures.

Kusu oil thus obtained forms a white crystalline mass at the ordinary temperature. It has a slight aromatic odour which recalls that of cocoanut oil. When warmed, it melts to a pale yellow liquid.

Specific gravity (at 25°C.)	0·9267
,, ,, (at 100°C.)	0·8760
Melting point	22·8°C
Acid value	4·70
Saponification value	283·76
Iodine value (Wijs)	4·49
Refractive index (at 25°C)	1·4517
Reichert-Meissl value	0·53

In the determination of Hehner value, 2 litres of washing water were employed and a value of 81·8 was obtained; but the soluble fatty acids had not yet been completely removed.

With the mixed acids (obtained by using 10% common salt solution for washing) the following values were determined:

Specific gravity (at 100°C)	0·8412
Melting point	21°C.
Neutralisation value	292·83
Mean molecular weight	191·57
Iodine value (Wijs)	5·07

The saponification value of kusu oil is very high, and indeed excells that of cocoanut oil. On the contrary, the Reichert-Meissl value is very small. It may be inferred from its properties, that the chief constituent of the oil is likely to be laurin.

The oil resembles cocoanut oil, and may probably be used for the same purposes as the latter oil.

Inukusu Oil.

This oil is obtained from the fruit of *Machilus Thunbergii S. et Z.*[1] (*Lauraceae*).

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[1] The tree is also known by the name of "*tabu-no-ki.*"

A specimen of the fruit from Fukuoka consisted of dark grey spheres larger than those of the kusu. They are about 0·8 centimeter in diameter and weigh about 0·22 gram per fruit. The analysis of the sample is as follows:

Moisture	4·28%
Oil	65·09 „
Ash	1·13 „

An oil prepared at Fukuoka was used for the examinations. It is a brownish yellow oil which at low temperatures deposited crystalline masses.

The chief values were determined with the following results:

Specific gravity (at 25°C)	0·9347
Acid value	19·31
Saponification value	241·39
Iodine value (Wijs)	66·08
Refractive index (at 25°C.)	1·4646
Reichert-Meissl value	2·05

The oil resembles kusu oil, but its lower saponification value and higher iodine value point to the presence of the glycerides of unsaturated higher fatty acids.

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On Japanese Lacquer.

By

Kisaburō Miyama, *Kōgakushi.*

I. The Principal Constituent of the Lacquer.

Japanese lacquer or *Urushi* is a milky juice exuding from the trunk of the lacquer-tree or *Urushihaze* (Rhus vernificera, D. C.) and is very widely employed in the manufacture of lacquered wares in Japan. The milky juice called raw lacquer or *Ki-urushi*, loses its moisture on exposure to the sunlight or on warming, and becomes a brown oily liquid.

For practical purposes the moisture of the raw lacquer is expelled and oils, coloring matter etc. are added; the lacquer thus obtained is called finished lacquer or *Seishi-urushi*.

The raw lacquer consists mainly of a brown liquid, gum arabic, enzymic nitrogenous matter and moisture. The brown liquid, the predominant and most important constituent of the lacquer, was named *Urushic acid* by O. Korschelt and H. Yoshida[1] who investigated the subject some twenty years ago. According to these investigators, the brown liquid is a monobasic acid of the formula $C_{14}H_{18}O_2$, and is oxydised to oxyurushic acid $C_{14}H_{18}O_3$ on drying. However, the series to which this acid belongs has not yet been determined, and furthermore its behavior is different from that of acids. As a matter of fact, characters common to

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[1] H. Yoshida: "Chemistry of Lacquer (Urushi). Journal Chem. Soc.,' 1883. P. 472.

organic acids are not found in it, and so it can not be proved to be an acid.

Recently, A. Tschirch and B. Stevens suggested that it was a resin of the empirical formula $C_{102}H_{138}N_2O_{19}$ and named it *Urushin.*

However, the presence of nitrogen is very doubtful, and also the sample analysed by them seems to have been already oxydised by drying, because its oxygen content is far different from that of urushic acid, and such an analytical result, I believe, can not be expected from any sample of carefully prepared urushic acid.

Therefore, the problem of ascertaining the chemical constitution of the principal constituent has been especially interesting to me.

The Composition and the Molecular Formula of the Lacquer.

The principal constituent, the so-called *Urushic acid* is prepared best from the raw lacquer by extracting with absolute alcohol and removing the alcohol by distillation. The brown liquid thus obtained, reddens a blue litmus paper; but after repeated washing with a large quantity of hot water, the acid reaction is transferred to the washings and the brown liquid no longer reddens the test paper. The washings contain a little acetic acid and an extractive matter which resembles gum arabic in its appearance but differs from the latter in its composition and solubility in absolute alcohol.

Old samples of the brown liquid are partly changed to a thick liquid insoluble in petroleum ether. Therefore, the constituent for investigation was purified by repeated washing with a large volume of boiling water, and by extracting with ten times its volume of petroleum ether.

It is not certain that the constituent thus purified is a strictly pure compound, but, since no other method of purifying has been found it is assumed to be a pure compound.

The raw lacquer for investigation was collected in the province of *Yamato* under special supervision, and was warranted to be free from any admixture.

The principal constituent consists of carbon, hydrogen, and oxygen; and its percentage composition is as follows:

	C	H	O
(1)	78·48	9·55	11·97
(2)	78·03	9·65	12·32
Mean	78·25	9·60	12·15
$C_{34}H_{50}O_4$ requires	78·10	9·65	12·25

The molecular weight determined by Raoult's method is 509.

From the above results, it appears that the molecular formula of the constituent corresponds to $C_{34}H_{50}O_4$.

The General Behavior of the Principal Constituent.

The important modes of behavior of the principal constituent are as follows:

1. The principal constituent is slightly heavier than water, (density at 15°C is 1·0025) and refracts the light considerably, (refractive index at 20°C is 1·5335,).
2. It does not freeze even at -20°C, and can not be distilled without decomposition even under a diminished pressure.
3. It is easily hardened by means of litharge, manganese dioxide and some other oxydising agents, even in the absence of the enzymic nitrogenons matter. Also, it hardens at a tempera-

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2. A. Tschirch and A. B. Stevens: "Japan lacquer. Arch. Pharm.," 1905. 243. 504—553.

ture above 96°C in the absence of the enzyme or any other oxydising agent.

4. It increases in viscosity and in intensity of colour on long storing.

5. It is only slightly soluble in aqueous caustic potash solution, but is soluble in alcoholic potash.

6.* With silver nitrate, the alcoholic solution of the constituent throws a preciptate and forms a silver mirror on warming.

7.* With lead acetate, a lead salt is precipitated.

8.* With caustic potash, a deep brown colour is produced.

9.* With strong nitric acid, a nitro-compound is produced with a violent reaction.

10.* Bromine is considerably absorbed by the constituent.

11. With ferric chloride, the alcoholic solution gives a black colour; and the highly diluted solution gives a green colour which turns red on the addition of sodium carbonate.[1]

12. With ammoniacal silver nitrate solution, a beautiful silver mirror is formed in the cold.[2]

13. With barium hydroxide, a green precipitate is produced.

14. With hydroiodic acid, it becomes a gummy mass, and its alcoholic solution gives a green colour with ferric chloride.

15. It does not reduce Fehling's solution even at a high temperature.

16. It does not decompose metallic carbonates.

17. Acetic anhydride or acetyl chloride produces an acetyl-derivative; and benzoyl chloride, a benzoyl-derivative.

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* The reactions 6—10 are the writer's observations, priority being given to former investigators.

[1] The reaction common to o-dihydric phenols.

[2] The reaction common to aldehydes, polyhydric phenols, and a few carbohydrates.

18. With phenylisocyanate $C_6H_5N:CO$, a phenyl carbamate is produced.

19. With benzyl chloride $C_6H_5CH_2Cl$, and sodium ethylate, a phenolic benzyl ether is produced.

20. By dry distillation more than two kinds of phenol are produced.

The State of Oxygen in the Principal Constituent.

In order to determine to what class of compounds the principal constituent belongs, I first proceeded to ascertain the state of the oxygen existing in the substance.

Absence of Carboxyl Group. About 0·2 gram of the substance was shaken with 20 c.c. of water and titrated with the decinormal caustic potash solution as usual; but it became alkaline on the addition of only one or two drops of the solution.

In the same way, the alcoholic solution of the substance did not absorb caustic alkali.

Lastly, the substance was shaken with sodium carbonate solution (10%) and filtered; the filtrate acidified with hydrochloric acid and shaken with ether; but there was no organic acid found in the ethereal solution.

Therefore, the presence of the carboxyl group can not be positively proved.

According to O. Korschelt and H. Yoshida, the constituent yields the lead salt $Pb(C_{14}H_{17}O_2)_2$ with lead acetate, and this was taken as a reason for an acid. However, the lead salt is not of a definite composition and the percentages of lead and oxygen vary with the condition of preparation.

Several samples of the lead salt, which were precipitated from the alcoholic solution, with an excess of lead acetate, and well washed with absolute alcohol, were analysed with the following results:

	C	H	O	Pb
No. 1 (vacuum-dried)	47·70	5·63	8·97	37·70
No. 2 (air-dried)	47·07	5·69	9·96	37·28
No. 3 (,,)	44·97	5·20	8·81	41·02
No. 4 (,,)	43·40	5·00	8·91	42·69
$Pb(C_{14}H_{17}O_2)_2$ requires	52·42	5·30	9·99	32·29

Since none of the above samples coincide with the formula $Pb(C_{14}H_{17}O_2)_2$ which was given by former investigators, it is difficult to prove the presence of carboxyl group by the composition of the lead salt.

Absence of Carbonyl Group. Since a substance that reduces silver from ammoniacal silver nitrate solution may be an aldehyde, the newly collected lacquer was tested for aldehydes, first with the fuchsin aldehyde reagent; next, with sodium bisulphite; but the results were negative.

Lastly the quantitative determination of the carbonyl group was carried out by heating a mixture of the constituent, phenylhydrazine and Fehling's solution, but no carbonyl was found.

These results show that the constituent is not an aldehyde or a ketone.

Presence of Hydroxyl Group; the Formation of the Acetyl- and Benzoyl-derivatives. The principal constituent is easily *acetylated* by means of acetic anhydride or acetyl chloride. When 5 parts of the substance is mixed with 2 parts of dried sodium acetate and 8 parts of acetic anhydride, and boiled for 2–3 hours, the acetyl-derivative is produced. The derivative is a yellow neutral liquid, slightly soluble in cold alcohol and gives no reaction with ferric chloride or lead acetate. It is saponified by caustic alkali or

barium hydroxide and gives off a considerable quantity of acetic acid.

The analysis of the derivative and the determination of its acetyl value were carried out with the following results:

		C	H	O	*Acetyl value*
Acetyl-derivative or Acetic ester	(1)	73·12	8·34	18·54	320
	(2)	72·82	8·46	18·72	
	(3)	72·36	8·72	18·92	
	Mean	72·77	8·51	18·72	
$C_{34}H_{46}(O.C_2H_3O)_4$	requires	72·98	9·49	18·52	325

The acetyl value was determined by saponifying the derivative with alcoholic potash, and distilling the saponification product with phosphoric acid.

The molecular formula of the derivative corresponds to $C_{34}H_{46}(O{\cdot}C_2H_3O)_4$ and shows that the original substance is of the formula $C_{34}H_{46}(OH)_4$.

The Iodine value of the derivative is 123.

Benzoylation of the principal constituent can be effected with ease by the Shotten-Baumann method and a fragrant neutral derivative of a yellow colour which closely resembles the acetyl-derivative in its behavior, is produced.

This derivative was analysed with the following results:

		C	H	O	*Benzoyl value*
Benzoyl-derivative or Benzoic ester	(1)	78·97	7·53	13·55	239.
	(2)	78·67	7·17	14·16	
	Mean	78·80	7·35	13·81	
$C_{34}H_{46}(O{\cdot}C_7H_5O)_4$	requires	79·05	7·17	13·78	241·6

Thus, the molecular formula of the derivative corresponds to $C_{34}H_{46}(O{\cdot}C_7H_5O)_4$.

From the above experiments, it appears that the oxygen in the principal constituent is in the state of the *hydroxyl* group.

The Proof of the Principal Constituent being a Phenol.

Nitration. The principal constituent is easily nitrated with strong nitric acid as in the case of many aromatic compounds; therefore it probably belongs to the aromatic series. The number of nitro-groups in the nitro-derivative varies with the condition of nitration; and by long boiling with strong nitric acid, the derivative, which at first is an orange solid, becomes a yellow liquid and large crystals of oxalic acid separate out after standing.

Bromination. When an excess of bromine is dropped into the ethereal solution of the constituent, there is formed a brominated product.

The pure brominated product is a reddish solid and gives the following composition:

		C	H	O	Br
Brominated Product (vacuum-dried)	(1)	27·11	3·10	4·40	
	(2)	27·29	3·12	4·20	65·39
	Mean	27·20	3·11	4·30	
$C_{24}H_{46}Br_{12}O_4$	requires	27·59	3·14	4·33	64·94

Thus the product is of the formula $C_{24}H_{42}Br_{12}(OH)_4$ and its molecule contains eight atoms of the added bromine and four atoms of the substituted bromine. The number of the substituted bromine atoms varies with the condition of bromination, but the number of the added bromine atoms remains unaltered.

100 parts of the principal constituent absorbs 293 parts of iodine by Wijis' method; therefore the molecule of the constituent takes twelve atoms of iodine which seem to be partly added and partly substituted.

Now, the brominated product $C_{24}H_{42}Br_{12}(OH)_4$ may be concerned as a derivative of the hydrocarbon $C_{24}H_{58}$ which must be a saturated compound. Since the hydrocarbon $C_{24}H_{58}$ differs greatly from the general formula C_nH_{2n+2} of the saturated hydrocarbons, it must be of the aromatic series.

Therefore, it may be concluded that the constituent under investigation belongs to the aromatic series, and must be either a phenol or an aromatic alcohol.

Phenolic Character. The alcoholic solution of the constituent gives a colour varying from green to brown with caustic alkali; this reaction shows that the constituent should be sought among the phenols. With ferric chloride, the solution, highly diluted, gives *a green colour,* which *turns red* on the addition of sodium carbonate; this is a reaction common to *o*-dihydric phenols. Also, the constituent produces a beautiful silver mirror with ammoniacal silver nitrate solution in the cold, as in the case of polyhydric phenols.

Dry distillation. Dry distillation of the constituent produces water, unsaturated hydrocarbons, and a heavy oil, and about thirty per cent. of the constituent remains as a pitch.

The heavy oil gives fairly phenolic reactions, and is a mixture of phenols and unsaturated hydrocarbons; it contains at least two kinds of phenols, one soluble, and the other insoluble in water.

With ferric chloride, both the alcohol solution highly diluted, of the insoluble phenol, and that of the soluble phenol give a green colour which turns red on the addition of sodium carbonate.

With lead acetate, they throw down a precipitate of greyish lead salt.

They reduce silver from the ammoniacal silver nitrate solution and absorb a considerable quantity of bromine, as the original substance does.

Although they are almost colourless when distilled, they turn brown on exposure to the atmosphere. In the presence of alkali, they turn black instantly. The insoluble phenol closely resembles the original substance, and it is decomposed into phenols and hydrocarbons again, when subjected to fractional distillation.

Therefore it is very difficult to obtain the phenols in the pure state.

The Formation of the Phenolic Ether. Benzyl ethers of phenols are, in general, produced by heating phenols with benzyl chloride ($C_6H_5CH_2Cl$) and sodium ethylate for 3–4 hours.

Since the constituent under investigation behaves as a phenol, I proceeded to prepare its benzyl ether by the above method, and obtained a neutral phenolic benzyl ether.

The ether is a yellow liquid with the density 1·014 at 27°C, soluble in ether and hot alcohol, and gives no reaction with ferric chloride, or caustic alkali. Like other phenolic ethers, it is not saponified by alcoholic potash.

The ether was analysed with the following results:

		C	H	O
Phenolic ether	(1)	84·34	8·44	7·22
	(2)	84·48	8·46	7·06
	Mean	84·41	8·45	7·14
$C_{34}H_{46}(O{\cdot}C_6H_5CH_2)_4$	requires	84·30	8·45	7·25

Therefore, it is a phenolic ether of the formula $C_{34}H_{46}(O{\cdot}C_6H_5CH_2)_4$, and its formation is explained by the equation:

$$C_{34}H_{46}(OH)_4 + 4C_6H_5CH_2Cl + 4C_2H_5ONa =$$
$$C_{34}H_{46}(O{\cdot}C_6H_5CH_2)_4 + 4NaCl + 4C_2H_5OH$$

The iodine value of the ether is 114.

From the above experiments, it appears that the constituent is *a phenol.*

Summary.

The principal constituent of the lacquer is *an aromatic compound* containing carbon, hydrogen, and oxygen, the latter existing in the state of *hydroxyl.*

It shows phenolic character in its behavior and contains at least two *phenolic hydroxyl* groups in the ortho-positions.

It is *an unsaturated compound* and absorbs eight atoms of halogens in the molecule.

Therefore, the principal constituent is *a polyhydric phenol* containing unsaturated radicles in its side-chains, and I propose for it the name of *Urushiol.*

II. Some Important Properties of the Lacquer.

The Composition and the Quality of the Raw Lacquer.

The raw lacquer consists of moisture, urushiol, gum arabic, nitrogenous matter and some added oils. The compositions of the specimens of superior lacquer are as follows:

Specimen	Moisture	Urushiol	Gum	Nitrogenous Matter
No. 1	9·32	86·97	2·46	1·25
No. 2	12·21	80·63	5·69	1·47
No. 3	10·94	84·53	3·25	1·28

The specimens of raw lacquer found in commerce vary to a considerable extent, their quality depending mainly on the locality and the period in which they are collected.

The specimens collected from the same lacquer-tree at different periods gave the following compositions:

	Moisture	Urushiol	Gum	Nitrogenous matter
Sakari-urushi (from Jul. 11th to Aug. 31st.)	17·81	77·63	2·62	1·94
Urame-urushi (from Sep. 21st to Oct. 10th.)	22·61	70·20	4·74	2·45
Tome-urushi (from Oct. 11th to 31st.)	23·30	66·66	7·57	2·47
Seshime-urushi (Collected from the branches during Nov.)	27·62	64·14	6·46	1·78

Sakari-urushi which is collected during the heat of summer is of the best quality and when freed from moisture, it contains urushiol 94·5 per cent.

The lacquers imported from China and India are far inferior in quality as is shown in the following analyses:

	Moisture	Urushiol and oils	Gum	Nitrogenous matter
Chinese lacquer (superior)	20·37	70·02	7·72	2·34
Chinese lacquer (middle)	30·74	55·88	11·78	1·60
Chinese lacquer (inferior)	36·85	36·88	23·55	2·72
Indian lacquer (middle)	33·38	26·39	37·78	2·45

Among the constituents of the lacquer, the most important is urushiol, and the more of this is contained, the better is its quality. Gum arabic is of no value, and the more of this there is, the more inferior is the quality. The nitrogenous matter is a constituent necessary for the drying of the lacquer, which, without it, is impossible. It differs from albumen in its composition, and consists mainly of an oxydation enzyme.

The Drying of the Lacquer.

The lacquer spread in a thin layer dries rapidly in a damp atmosphere and hardens to a lustrous coating which is extremely stable and resistant to various solvents and chemicals.

For the drying of the lacquer, the presence of moisture and a temperature between 10°—30°C, are necessary; for this reason, the lacquer dries more slowly in a dry season than in a wet season; and in the winter than in the summer.

The drying of the lacquer is much retarded by warming to a temperature above 50°C, and its drying quality, is entirely suspended by keeping it at a temperature of 70°—80°C; in this respect, the drying of lacquer differs from that of drying oils.

Drying at a high temperature, however, by no means requires the presence of moisture or enzymic nitrogenous matter.

In a steam-oven or an air-oven with a temperature above 100°C, the lacquer dries within 4—5 hours, and the higher the temperature is, the more rapidly it dries. For instance, at 150°C it dries within thirty minutes, and at 180°C within ten minutes. Lacquer which has lost the drying quality, or even urushiol which does not dry by itself, dries readily at a temperature above 100°C.

Absorption of Oxygen in Drying.

The Increase in Weight of the Lacquer on Drying at an Ordinary Temperature. When the lacquer dries, its weight is more or less increased by the absorption of oxygen.

However, the increase of weight during the period in which the lacquer is hardening to a solid film is not considerable; but the increase continues at least for three months and becomes a considerable amount.

This fact is shown by the following experiments:

The raw lacquer spread in a thin layer inside a beaker was dried in a desiccator overnight and the lacquer thus freed from moisture was weighed; next, it was dried (*hardened*) in a damp cupboard for two days and after drying in a desiccator overnight, was weighed again.

The same operation was repeated several times and the increase of weight was observed.

Lacquer	0·6066 gram
,, after drying for 2 days	0·6167 ,,
,, after drying for 90 days more	0·6415 ,,

increase of weight in 92 days=0·0349 gram
=5·75 per cent of the lacquer.

The lacquer, completely hardens in the first two days, but the increase of weight does not cease then.

Change in Composition on Drying at an Ordinary Temperature. The Lacquer free from moisture, and a coating of lacquer dried for ninety-two days were analysed with the following results:

	C	H	O	Ash
Lacquer	75·35	9·22	15·00	0·43
Lacquer-coating	70·96	8·82	19·80	0·42
Lacquer+5·75% Oxygen *(calculated)*	71·25	8·72	19·63	0·40

From these results, it is seen that, in drying at an ordinary temperature, there is no change other than the absorption of oxygen.

The Increase in Weight of the Lacquer on Drying at a high temperature. The weighing was conducted as in the above experiment, and the drying was effected in a steam-oven at 96°C, and in an air-oven at 120°C successively.

Lacquer	0·6334
,, after drying for 6 h. at 96°C	0·6433
,, after drying for 63 h. more at 96°C	0·6767
,, after drying for 71 h. more at 120°C	0·6782

Increase of weight in 140 h.=0·044 gram
=7·07% of the lacquer

The lacquer completely hardens in the first six hours, but the absorption of oxygen is rather considerable after the lacquer has hardened to a solid film.

Change in Composition on Drying at a High Temperature. The lacquer free from moisture, and a lacquer-coating obtained by drying for one hundred and forty hours at a high temperature were analysed with the following result:

	C	H	O	Ash
Lacquer	75·35	9·22	15·00	0·43
Lacquer-coating	66·61	7·19	25·72	0·48
Lacquer+7·07% Oxygen *(calculated)*	70·36	8·61	20·63	0·40

The composition of the lacquer-coating differs very much from the figures calculated, and it is seen that, in drying at a high temperature, not only the absorption of oxygen, but also a partial decomposition of the lacquer takes place. In this respect, drying at a high temperature differs from drying at an ordinary temperature.

Driers for the Lacquer and Some Other Substances Affecting the Properties of the Lacquer.

Notwithstanding urushiol, the principal constituent of the lacquer can not be dried in the absence of enzymic nitrogenous matter, it is easily dried by means of manganese peroxide, barium peroxide, magnesium peroxide, litharge, manganese hydroxide, potassium bichromate or manganese resinate.

Therefore, these salts serve as driers for the lacquer, but they can not be used for transparent lacquers, nor for coloured lacquers other than the black, because the lacquers are rapidly blackened by them.

The drying of the lacquer is very much injured by acids and alkalies, and some metallic salts such as sodium chloride and ferrous sulphate.

Lacquer is blackened by contact with metallic powders such as iron, zinc, lead, copper etc. except tin, silver, aluminium, gold and platinum. Most of the white pigments, such as zinc oxide, zinc sulphide, calcium carbonate, calcium sulphate, white lead etc. turn black when they are mixed with the lacquer.

Most of the organic lakes, also, are entirely changed by contact with the lacquer.

For these reasons, pigments for the lacquer are very much limited.

III. Technical Analysis of the Lacquer.

The Usual Method of Examination.

The value of lacquer is dependent upon the durability, the transparency, the colour, the lustre and the smoothness of the lacquer-coating, and upon the viscosity and the drying time of the lacquer.

The chemical analysis of the lacquer generally employed is as follows:

About 1 gram of the lacquer is dissolved in 30 c.c. of absolute alcohol, filtered through a tarred filter and well washed with absolute alcohol. From the filtrate and washings, the alcohol-soluble matter (a mixture of urushiol and oils) is determined after evaporating the alcohol. Next, the residue on the filter is washed repeatedly with boiling water; and from the washings and the

insoluble residue, the water-soluble matter (gum arabic) and the nitrogenous matter are respectively determined. The difference between the weight of the sample and the sum of the alcohol-soluble, the water-soluble, and the nitrogenous matter is taken as moisture.

The constituents of raw lacquer to be estimated are moisture, urushiol, gum arabic, nitrogenous matter and some added oils; and those of the finished lacquer are, in addition to the above, colouring matter, drier, and some other added substances.

Owing to the constant admixture of oils in the lacquer, it is desirable to have a complete method of separating the urushiol from the oils and other added substances. In the usual method of analysis, however, it is almost impossible to separate the urushiol from the oils, therefore I tried to find a new method of determining the urushiol.

A New Method of Determining Urushiol.

Urushiol is detected by the following tests:

> With ferric chloride, a highly diluted solution of urushiol gives a green colour which turns red on the addition of sodium carbonate.
>
> With ammoniacal silver nitrate, it forms a silver mirror in the cold.
>
> With barium hydroxide, the solution deposits a characteristic green precipitate.

Among these, the reaction with barium hydroxide is the most important, and is employed for the estimation of urushiol.

If a slight excess of a barium hydroxide solution is dropped into an alcoholic solution of urushiol, with the previous addition of a few drops of phenolphthalein solution, a green precipitate of barium salt is deposited, and the supernatant liquid is coloured pink. Therefore, urushiol may be determined volumetrically with a standard barium hydroxide solution.

The barium salt is more or less decomposed when water is present; for instance, if a titrated urushiol solution which is not yet alkaline, is diluted with a small quantity of water, it becomes fairly alkaline.

Thus the quantity of barium hydroxide absorbed by urushiol varies with the strength of the barium hydroxide solution and the quantity of alcohol used in the titration. The following table shows the results of the titration of 1 gram of urushiol with barium hydroxide solutions of various strengths.

Exp.	Alcohol used	$Ba(OH)_2$ Sol. required	Calculated as N. $Ba(OH)_2$ Sol.
(1)	250 c.c. abs. alcohol	7·0 c.c. N. Sol.	7·0 c.c.
(2)	250 ,, ,, ,,	24·6 ,, $\frac{N}{4}$,,	6·15 ,,
(3)	250 ,, ,, ,,	41·5 ,, $\frac{N}{8}$,,	5·19 ,,
(4)	250 ,, 97% alcohol	51·5 ,, $\frac{N}{10}$,,	5·15 ,,

From the above experiment, it appears that the barium hydroxide solution and the alcohol to be used in the titration must be of a definite strength, and the quantity of alcohol must be in a definite proportion relative to that of the urushiol to be titrated. In practice, it is convenient to use 200–250 c.c. of absolute alcohol for every gram of urushiol, and the quarter-normal barium hydroxide solution in the following way:

About 0·5 gram of the urushiol under examination is dissolved in 100-125 c.c. of absolute alcohol with the addition of a few drops of phenolphthalein solution, and titrated with the quarter-normal barium hydroxide solution, until the supernatant liquid gives a pink colour.

By this method, six samples of urushiol were titrated with the following results:

		Substance taken	$\frac{N}{4}$ $Ba(OH)_2$ Sol. required	Normal $Ba(OH)_2$ Sol. for 1 gram urushiol
Urushiol	(1)	0·2465 gram	6·05 c.c.	6·135 c.c.
	(2)	0·3051 ,,	7·50 ,,	6·146 ,,
	(3)	0·3074 ,,	7·55 ,,	6·138 ,,

	Substance taken	$\frac{N}{4}$ Ba $(OH)_2$ Sol. required	Urushiol found
Mixture of {*urushiol* 69·50 *linseed oil* 30·50}	0·1777 gram	3·05 c.c.	69·93%
,, ,, {*urushiol* 59·87 *mixed oil* 40·13}	0·4923 ,,	7·25 ,,	59·95 ,,
Impure Urushiol from an inferior lacquer	0·3074 ,,	6·65 ,,	91·16 ,,

Since the first three experiments show that 1 gram of urushiol requires 6·14 c.c. of the normal barium hydroxide solution, the percentages of urushiol in the impure samples are calculated from the results of titration according to the formula:

$$\text{Urushiol} = \frac{V}{6{\cdot}14} \times 100$$

where V = volume in c.c. of the normal barium hydroxide solution required for 1 gram of the samples.

In the above titration, the oils remain indifferent and the quantitative determination of urushiol is effected.

The quantity of the barium hydroxide absorbed by urushiol increases as the strength of the barium hydroxide solution increases, and gradually approaches the theoretical number calculated from the equation:

$$C_{34}H_{50}O_4 + 2Ba(OH)_2 = C_{34}H_{46}O_4Ba_2 + 2H_2O$$

But, it falls to one half of this number when the precipitated barium compound is decomposed with a large quantity of hot water and then titrated back with a standard hydrochloric acid.

The composition of the barium compound, precipitated from the urushiol solution with barium hydroxide, varies with the quantity of the precipitant, and the condition of preparation; but certainly the salt is an urushiolate which has absorbed a large quantity of oxygen. For instance, the compound precipitated from the urushiol solution with 75 per cent of the theoretical amount of barium hydroxide, and washed with absolute alcohol gives the following composition:

C	H	O	Ba
51·40	6·15	16·74	25·71

These figures correspond to the formula $Ba_{1.5}C_{34}H_{47}O_{8.3}$, showing that the composition of the urushiolate is indefinite.

The urushiolate absorbs oxygen rapidly, and the original urushiol can not be obtained on decomposing it with acids.

When the newly precipitated barium urushiolate is thrown into a large quantity of water without delay, and well washed, a different kind of barium salt is obtained, which gives the following composition:

		C	H	O	Ba
No. 1. (air-dried)	(1)	56·44	6·71	17·25	19·60
	(2)	56·15	6·64	17·56	19·65
	Mean	56·30	6·67	17·40	19·63
No. 2. (air-dried)	(1)	56·06	6·60	18·07	19·27
	(2)	56·00	6·69	18·02	19·29
	Mean	56·03	6·65	18·04	19·28
$BaC_{34}H_{48}O_8$ requires		56·54	6·70	17·73	19·03

This barium salt gives an almost definite composition corresponding to the formula $BaC_{34}H_{48}O_8$, and contain eight atoms of

oxygen in one molecule, that is to say, twice the number of oxygen atoms contained in urushiol.

Barium urushiolate is insoluble in petroleum ether and cold alcohol but soluble in chloroform.

Analysis of the Raw Lacquer.

Moisture. About 1 gram of lacquer filtered through a cotton cloth, is weighed into a beaker, and dried on a water-bath until the liquid becomes transparent, and then put into a steam-oven for half an hour. The loss of weight gives the amount of moisture.

Urushiol. Lacquer free from moisture is dissolved in 10 c.c. of absolute alcohol, and after standing for half an hour transferred to a tarred filter, and washed with absolute alcohol. The filtrate and washings are divided into two equal parts, one of which is used to determine the alcohol-soluble matter, and the other to titrate urushiol with the standard barium hydroxide solution.

Oils. The difference between the alcohol-soluble matter, and urushiol gives the amount of oils

Gum arabic. The residue on the filter is extracted with boiling water and the aqueous solution is evaporated to dryness and weighed.

Nitrogenous matter. The insoluble residue on the filter is nitrogenous matter; it is weighed after drying.

Analysis of the Finished Lacquer.

Moisture. Moisture is determined in the same manner as in the case of raw lacquer; but it is better to take about 2 grams of the sample.

Urushiol. Lacquer free from moisture is dissolved in 20 c.c.

of absolute alcohol and after standing for an hour, is transferred to a tarred filter and the alcohol-soluble matter is determined.

In the case of black lacquer, one or two drops of the half normal hydrochloric acid must be added, or the lacquer is not easily dissolved.

The alcohol-soluble matter is washed first with hot water,[1] next with warm sodium carbonate solution,[2] and then with hot water again, and redissolved in alcohol.

The alcoholic solution is divided into two equal parts, one of which is titrated with the standard barium hydroxide solution in order to estimate the urushiol, and the other is evaporated on a water bath and weighed to determine the sum of urushiol and oils.

Oils. The amount of oils is obtained by subtracting that of urushiol from the sum of urushiol and oils.

Gum arabic. This is determined in the same way as in the case of raw lacquer, but all water-soluble admixtures such as honey and maltose come under this head.

Nitrogenous matter. This is determined in the same way as in the case of raw lacquer; but all insoluble admixtures come under this head.

Ash. Ash is determined by igniting 1 gram of the lacquer in a basin. Inorganic substances contained in the ash, often suggest the driers or some of the admixtures in the lacquer.

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[1] From the solution, water-soluble matter as glycerine may be determined.

[2] From the solution, resinous acid and its acid value may be determined.

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