

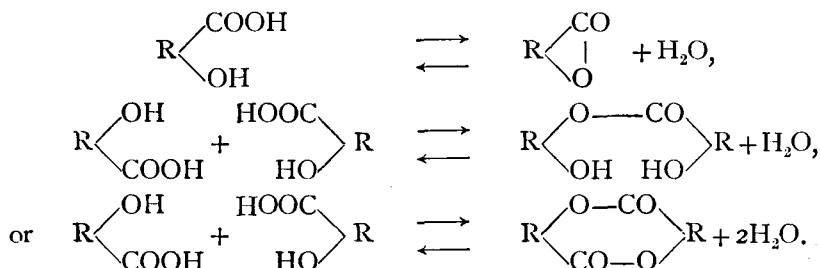
On Kakishibu, III. Constitution of Shibuol, II.¹

By

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In the first communication on this subject² were described the methods for isolating shibuol in a fairly pure state from the kakishibu by converting it into the insoluble form, which, in the opinion of the authors, would result from the condensation of the COOH and OH groups present in the molecule of shibuol, forming the anhydride from one molecule or two molecules united one with another in either of two ways thus:



The chemical study of the nature of this process which was regarded by the authors to be comparable to the coagulation of proteins by heat or the action of chemicals, was very important not only because it has a direct connection with the problem of the chemical constitution of shibuol, but from the view point of plant physiology, with the desire to find some explanation for the ripening process of the kaki-fruit.

The coagulation of the soluble shibuol by means of chemicals into the insoluble form is caused by the inner condensation of the molecule, it

¹ The expenses of this investigation were shared for the advancement of natural sciences by the Department of Education.

² These Memoirs, 7, 15 (1923).

seems much more probable that such a molecule, whatever the mode of union, would of course form a compound with bases or water with much more difficulty than the soluble shibuol which had not undergone such internal neutralization, before since the dissociation could occur, the ring-formation would have to be opened up. When heat was applied to the mixture of insoluble shibuol and water or bases, it must result in shifting the equilibrium in the direction of hydrolysis, and thus soluble shibuol would be prepared in a pure state, when chemical change had not occurred in the molecule by the treatment.

1. Action of Superheated Water on Insoluble Shibuol.

3 grms. of the insoluble shibuol with 30 c.c. of pure water were heated in a sealed tube at 125–130 for 10 hours, and it was converted completely into a soluble form which was then divided by the difference of their solubility in cold water into two parts: the soluble and the insoluble forms.

The analysis of the samples was made with the following results:

	Cold water-soluble form.	Cold water-insoluble form.
C.	57.70; 57.74.	57.56; 57.29.
H.	4.13; 4.42.	3.82; 3.88.
Ash.	10.27; 10.20.	0.2; 0.2.

The only difference between the two forms is, as may be seen in the table, in the ash content, the more soluble form contains very much more ash than the less soluble one, and this ash is composed mostly of calcium.

These facts above-mentioned lead naturally to the conviction that free OH and COOH groups exist in the molecule of the soluble shibuol and by the binding together of these groups shibuol becomes insoluble and shows no more an astringent taste.

In the ripening process of the kaki-fruit, so far as we have studied, it was decided shibuol was not actually used up in the process, but remained in the ripe fruit, and in part at least, with the transformation of the soluble form into the insoluble, which was accomplished as we have already had strong reason to believe, by the dehydration of the molecule.

This conception for the ripening of the fruit was further supported by the fact that the insoluble shibuol, as we expected, can be converted into the soluble form by digesting it with lime water or calcium carbonate, while in the digestion with a strong alkaline solution there occurs complete

disruption of the molecule with the formation of gallic acid and phloroglucinol.

Accordingly, with regard to the physiological signification of plant ash, the authors are inclined to think that at least some of the mineral matters in the fruit may play an important part in keeping shibuol in soluble form in the plant tissues, and the salts of shibuol which occur in the fruit and which with free soluble shibuol serve to impart to it the astringent taste, are decomposed by the action of the organic acids which develop, as the fruit ripens, into the salts of the acids and the shibuol which subsequently changes into the insoluble form.

2. Action of Dilute Acids on Shibuol.

The following experiment was undertaken with the expectation of finding, if possible, some explanation for the physiological significance of plant acids in the fruit.

1 gram of the soluble shibuol dissolved in 50c.c. of water, left to stand after mixing with 20c.c. of dilute hydrochloric acid (1:2) for 24 hours at ordinary temperature, was partly decomposed into gallic acid and partly transformed into an insoluble substance.

The latter substance separated from the mother liquor, washed with cold water, dried, and then treated with acetic ester, resulted in 0.02 grams of the ester-soluble substance and 0.6 gram of an insoluble one.

The insoluble residue was found by means of boiling with 94% alcohol to be composed of phlobaphene and the insoluble shibuol.

They were analysed with the following results:

	Phlobaphene.	Insoluble shibuol.
C.	60.70	57.00; 56.81.
H.	4.37	3.63; 3.61.
Ash.	5.28	— —

From the acidic mother liquor separated from the insoluble shibuol and phlobaphene, 0.01 gram gallic acid was isolated by extracting it with ether, which was confirmed by determination of its melting point (238°) and also by its chemical reactions toward ferric chloride and a caustic soda solution.

The fact above-mentioned, indicates that the partial decomposition and polymerisation of shibuol in the dilute mineral acid solution take place at ordinary temperature, though the velocity of these reactions is very slow under these conditions.

The action of heat on the mixture of shibuol and mineral acid was mentioned briefly in the first communication with the formation of phlobaphene.

It was analysed after being purified from a hot alcohol solution :

C.	60.08 ;	59.87.
H.	4.02 ;	3.98.
Phloroglucinol- content.		26.8.

The yield of phlobaphene and gallic acid formed from shibuol by the action of dilute sulphuric acid was determined as will be seen in the following analysis :

Shibuol.	3% H ₂ SO ₄ .	Time for boiling.	Phlobaphene. %	Gallic acid. %
5 grm.	100 c.c.	2 hours.	96.	2.
5 "	100 "	3 "	94.	1.6.

As a matter of fact, the formation of phlobaphene from shibuol by the action of dilute mineral acids was usually accompanied with that of gallic acid. Soluble shibuol, therefore, suffers, first, the dehydration, in the course of digestion with a mineral acid, to condense into the anhydride which then gives rise by cleavage of the molecule to gallic acid on the one hand, and on the other to phlobaphene by further condensation, and the latter in turn was broken down into gallic acid and phloroglucinol.

When 1 grm. phlobaphene was digested with 30 c.c. water in a sealed tube at 140–145, it yielded a substance with 0.1 grm. of gallic acid (*M. p.* 238°), which was analysed with the following results :

C=60.26 ; 60.64. H=3.35 ; 3.32. Ash=0.03 ; 0.06.

Gallic acid, thus isolated, was analysed.

C=48.84 ; H=3.45. The theory requires C=49.41 ; H=3.52 for C₆H₂(OH)₃·COOH.

Phlobaphene was also obtained from shibuol by the action of oxalic acid and also by treating it with magnesium amalgam in an alcoholic hydrochloric acid solution, and these reactions were similar to those of the formation of anthocyan from flavonols and of flavonols from chalcon and accordingly it was borne in mind that the constitutional relation between phlobaphene and shibuol should hold some analogy to that observed between anthocyan and flavonol and also between flavonol and chalcon.

Such analogy was also noticed in the tinctorial property of these natural colouring matters ; the detail will be described in another chapter.

These facts suggest the notion to the authors that phlobaphene

contains oxonium oxygen resulting from the removal of a water molecule from two hydroxyl groups, as we see in the formation of flavonols from chalcones, through which two benzene rings linked together form essentially a complex nucleus of the colouring matter.

The latter idea seems much more probable when referred to the fact that when the hydroxyl groups in shibuol were acetylated, the acetyl-shibuol gave no more phlobaphene by the action of mineral acids.

When the analytical figures of phlobaphene were compared with those of the "goma," there was noticed quite a resemblance between them, and moreover, the latter substance behaves toward boiling water, in a similar manner to the former, yielding gallic acid as a cleavage product.

3. Acetyl Shibuol.

Shibuol and phlobaphene, when hydrolysed with caustic potash, yield phloroglucinol and gallic acid, though the molecular proportion of the decomposition products was not the same in both cases.

Myricetin and delphinidin were also made up of practically identical decomposition units.

These natural pigments distributed in plant tissues, however, show quite different chemical properties in spite of being built up of the same constituents, due to the differentiation of the chemical structure of the complex nuclei in their molecules or the manner of linking of the constituents.

Acetylation of shibuol and phlobaphene was, therefore, undertaken in order to find the free hydroxyl group in their molecules, by which to ascertain the manner of linking of the two phenolic constituents.

The usual method for acetylation by means of acetic anhydride or acetyl chloride in presence of caustic soda or pyridine could not be employed in this particular case. The authors, however, have succeeded after many trials, in getting acetyl shibuol by means of acetic anhydride under heat with or without pressure, according to the method by Kostanecki and Tambor.¹

The reaction product washed with water to remove any adhering acetic acid, was treated with acetone or acetic ester, by which it was divided into the soluble compound and the insoluble one, and they were analysed after drying to constant weight.

The acetyl estimation following the method proposed by Freudenberg

¹ Ber. D. Chem. Ges. **35**, 1868 (1902).

and Harder¹ was carried out with both samples and the results are shown in the tables, I, II.

Table I.
Acetylation by boiling.

Shibuol. gm.	Acetic anhydride. gm.	Time for boiling.	Temp.	Yield. gm.	%	Residue. gm.	Acetyl-group content.
6	60	15	boil.	0.5	8.3	—	4-Mol.
5	50	5	"	0.5	10.0	4.6	"
5	50	7	"	0.65	13.0	5.5	"
5	50+50 glacialacetic acid.	5	"	0.8	16.0	7.0	"
5	50	2	"	0.85	17.0	5.2	"
5	50	6	"	0.9	18.0	5.05	"
5	50	5	"	0.9	18.0	6.3	"
6	60	15	"	1.1	18.0	5.0	"
5	50	3	"	1.0	20.0	5.1	"
5	60	5	"	1.25	25.0	6.5	"
4	40	10	"	1.2	30.0	3.1	"

Acetylation under pressure.

3	30	3	140°	0.25	7.5	3.4	"
3	30	5	140°	0.33	11.0	3.7	"
5	50	5	145°	0.55	11.0	5.5	"
3	30	6	150°	0.45	11.6	3.1	"
3	45	6	155°	0.35	11.7	3.2	"
3	30	6.5	160°	0.55	18.3	3.1	"

Table II.

Acetyl shibuol, obtained by boiling.				Acetyl shibuol, obtained by heating under pressure.			
C.	H.	Ash.	Acetyl-value.	C.	H.	Ash.	Acetyl-value.
57.74	4.58	—	39.49	57.88	4.16	0.12	40.16
57.80	4.58	—	39.38	57.53	4.23	0.13	40.37
57.29	4.51	0.89	37.68	57.19	4.57	0.04	39.49
57.84	4.30	0.79	37.89	57.23	4.50	0.03	38.86

Two forms, as will be seen in the tables, show no evidence to distinguish them from each other in elementary composition and acetyl value, except the solubility in acetone, chloroform and acetic ester and the

¹ Lieb. Ann., **433**, 230 (1923).

ash-content, the more soluble form contains much more ash than the other.

Both forms of the acetyl shibuol have no definite melting point, are insoluble in caustic soda solution and yield no phlobaphene by contact with dilute mineral acids. When the acetyl shibuols are brought into prolonged contact with boiling water, the result is a removal of the acetyl group from the molecule.

The modifications of acetyl shibuol show a similar relation in solubility and ash-content with those observed in the shibuols.

4. Acetyl Phlobaphene.

Phlobaphene was also acetylated by means of acetic anhydride under the same conditions as those described in the acetylation of shibuol.

The reaction product thus obtained was observed as in the case of acetyl shibuol to be separated by means of acetone, chloroform or acetic ester into the soluble and insoluble forms in these solvents. They show the same elementary composition and the acetyl value as may be seen in the table, III.

Table III.

Acetyl phlobaphene, obtained by boiling.				Acetyl phlobaphene, obtained by heating under pressure.			
C.	H.	Ash.	Acetyl-value.	C.	H.	Ash.	Acetyl-value.
60.22	4.13	—	25.28	58.39	4.02	—	34.79
59.92	3.89	—	25.37	58.14	4.05	—	37.24

When, however, it was acetylated in an open vessel, it resulted in diacetyl phlobaphenes, whilst under pressure in a sealed tube it yielded the triacetyl derivatives as shown in the table, IV.

Table IV.

Acetylation by boiling.

Phlobaphene. gm.	Acetic anhydride. gm.	Time for boiling.	Temp.	Yield. gm.	Yield. %	Residue. gm.	CH ₃ ·CO-group. content.
4	40	3	—	1.3	32.5	2.6	2 Mol.

Acetylation under pressure.

3	30	10	140°	0.6	20.0	3.4	3-Mol.
3	30	6	140°	1.5	50.0	0.7	„

It was also noticed that acetyl phlobaphene when boiled with water for a long time becomes hydrolysed into phlobaphene and acetic acid.

5. Hydrolysis of Acetyl Shibuols.

When 1 grm. of soluble acetyl shibuol was heated with 30 c.c. of water at 150 for 10 hours, it was completely decomposed into shibuol and acetic acid, and the latter substance expelled by steam distillation from the reaction product was estimated volumetrically with standard barium hydroxide solution, the acetate thus obtained was analysed after transforming into the silver salt.

	Shibuol.	CH ₃ COOAg.	
		Found.	Calc.
C.	57.69; 57.73	13.87	14.37
H.	4.40; 4.36	2.05	1.80
Ash.	0.07; 0.08	—	—
Ag.	—	63.55	64.67

The insoluble acetyl shibuol when heated with super-heated water at 150, yielded shibuol and acetic acid, and they were analysed with the following results:

	Shibuol.	CH ₃ COOAg.	
		Found.	Calc.
C.	57.58; 57.56	14.29	14.37
H.	3.60; 3.69	1.90	1.80
Ash.	0.05; 0.05	—	—
Ag.	—	64.47	64.67

1 grm. of acetyl shibuol was also hydrolysed by means of magnesium oxide and water according to the directions of Schiff¹ and 0.5 grm. shibuol and 1 grm. silver acetate were obtained. They were analysed with the following results:

¹ Ber. D. Chem. Ges., 12, 1531 (1879).

	Shibuol.	CH ₃ COOAg.	
		Found.	Calc.
C.	57.79; 57.40	14.28	14.37
H.	3.83; 3.97	1.88	1.80
Ash.	0.04; 0.04	—	—
Ag.	— —	64.20	64.67

6. Hydrolysis of Acetyl Phlobaphene.

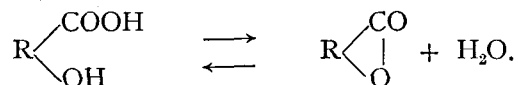
By heating acetyl phlobaphene with water in a sealed tube for 10 hours at 135°, it was completely hydrolysed into phlobaphene and acetic acid.

For confirmation, they were analysed with the following results :

	Phlobaphene.	CH ₃ COOAg.	
		Found.	Calc.
C.	59.52; 59.16	14.11	14.37
H.	3.31; 3.30	2.01	1.80
Ag.	— —	64.26	64.67

Summary.

1. The insoluble shibuol isolated by the precipitation method, was transformed inversely by the hydrolysis into the soluble one, and the conversion from one form to the other was ascribed to the following reversible reaction :



2. The coagulated shibuol or anhydride contains at least four hydroxyl groups, yielding tetraacetyl shibuol.

3. Shibuol when treated with dilute mineral acids, oxalic acid and magnesium amalgam in an alcoholic hydrochloric acid solution yields phlobaphene and some gallic acid, and the former was regarded as being formed by the removal of the water molecule from two hydroxyl groups in the shibuol molecule, resulting in an oxonium compound.

4. Phlobaphene when acetylated in a closed tube, gave triacetyl compounds, a fact which indicates presence of at least three hydroxyl groups in the molecule.

5. When acetyl shibuol and acetyl phlobaphene came into contact

with super-heated water, the acetyl group was completely removed from the molecules.

6. The mineral substances and organic acids in the fruit were discussed in detail from the view point of plant physiology.

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