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On Kakishibu, I. Constitution of Shibuol,* I.

By

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Kakishibu, in Japan, is prepared by treating unripe Kaki fruit of the astringent variety, crushed in a fresh state, with water at ordinary temperature and separating insoluble pulp from the extract¹.

It is used for the preservation of fish nets and fish lines, and wooden vessels and also to make paper water proof. Such technical applications of kakishibu, may be attributed to its containing a tannin-like substance in it.

The freshly prepared kakishibu is a light yellow turbid solution, and on standing in the open air, it turns gradually from yellowish brown to dark brown, by oxidation of the phenolic compound and forms a film on the surface of the solution.

The specimen used in the investigation was prepared on the 14th of Sept. 1922, specially from "AWASO", the astringent variety, grown at Seta, Shigaken, and started the experiments on the 26th of Oct. 1922.

It was a brownish-yellow turbid solution, sp. gr. 1.02. After many trials, the authors succeeded in getting a clear and transparent solution, by filtering through an asbestos mat in Buchner's funnel, using suction as usual.

The solution becomes light reddish-brown, by such treatment. It gives with ferric chloride solution, a bluish black precipitate which treated with dilute hydrochloric acid solution, changes into white. With

* The expenses of this investigation were shared by the Department of Education.

¹ Tsukamoto; Bull. Coll. Agri. Tokyo. 4. 329 (1902).

bromine water, a pale yellow precipitate was formed which indicates a phenolic compound presents in the solution.

This assumption was confirmed by preparing an azo-compound by coupling with diazobenzenechloride. The authors, therefore, chose the name "SHIBUOL" for the phenolic compound supposed to exist as a constituent of kakishibu.

Shibuol was precipitated as a lead salt from the solution, with both basic and neutral lead acetate solutions. This precipitate is soluble in dilute acetic acid. It formed a white amorphous compound with formaldehyde and a little dilute hydrochloric acid.

Shibuol was also precipitated as noticed by Tsukamoto¹. in a white amorphous compound which changed gradually to brown by treating with mineral and also acetic acids in a cold state. When treated with conc. sulphuric acid, it yielded a light yellow amorphous precipitate, which dissolved in the solution when gently warmed, and on boiling further a red-coloured amorphous precipitate, similar to "phlobaphene" was reproduced.

So far as we could ascertain, shibuol should be classified as phlobatannin².

Although many chemists³ have attempted to determine the constitution of shibuol, even isolation of the constituents has not yet been achieved owing to the difficulty of obtaining shibuol, in an even approximately pure state from kakishibu.

The authors, however, have succeeded in isolating shibuol in fairly pure condition from kakishibu, in the following ways:—

¹ Loc. cit.

² S. Komatsu and H Ueda: *J. Biochem.*, **2**, 298 (1923).

³ Tsukamoto: *Loc. Cit* 29; W. D. Bigelow, H. C. Gore and B. T. Howard: *J. Am. Chem. Soc.*, **28**, 688 (1906); F. E. Lloyd: *Biochem. Bull.*, **1**, 7 (1911); D. E. Clark: *Ibid.*, **2**, 412 (1912).

I. ISOLATION OF SHIBUOL.

1. BY PRECIPITATION WITH A MIXTURE OF ALCOHOL AND ETHER.

1000 c.c. of kakishibu prepared in clear and transparent state by filtration, were evaporated under reduced pressure to syrup, and treated with a mixture of alcohol and ether to precipitate shibuol. It was separated by filtration from the mother liquor, and washed with water, alcohol and ether successively, yielding 20 grms, corresponding to 2% of the kakishibu.

2. BY PRECIPITATION WITH GLACIAL ACETIC ACID.

2000 c.c. of the clarified shibu were evaporated under reduced pressure to a syrup, and treated with glacial acetic acid in a cold state. The precipitate, thus formed, was soluble in water. On being kept in contact with the acidic solution for a few hours at an ordinary temperature, it became insoluble. This insoluble substance was then washed with water, alcohol and ether. The sample, dried at 50° in vacuo, weighed 28 gm. (1.4% of the solution).

3. BY PRECIPITATION WITH CONC. HYDROCHLORIC ACID.

2000 c.c. of the kakishibu, evaporated to a syrup under reduced pressure as usual, were treated with cold conc. hydrochloric acid, and the precipitate formed, separated from the mother liquor, was washed with water, alcohol and ether, and dried at 50° in vacuo, yielding 21 gm. shibuol (1.2%).

4. BY PRECIPITATION WITH BASIC LEAD ACETATE SOLUTION.

1000 c.c. of the clarified kakishibu, were treated with a sufficient quantity of basic lead acetate solution to precipitate completely the shibuol. The insoluble lead salt, separated from the mother liquor, was washed with water. It was suspended in 500

c.c. water, and was decomposed by passing sulphuretted hydrogen gas over it. The filtrate, separated from lead sulphide, was light yellow. It was evaporated under reduced pressure almost to dryness, treated with 98% alcohol to precipitate shibuol, and filtered, washed with ether, and then dried in vacuo. It yielded 10.6 grm. (1% of the kakishibu).

The shibuol, when freshly isolated from the kakishibu by precipitation methods, was soluble in water. On standing in contact with the mother liquor for a few hours, however, it changed to the insoluble form. The shibuol thus obtained was soluble in alkali, but insoluble in water, alcohol, acetone and acetic ester.

It was dried at 100° under reduced pressure to constant weight, and analysed with the following results, calculated as ash free substance.

TABLE I.

Samples.	Carbon.			Hydrogen.			Ash. mean
	1.	2.	mean	1.	2.	mean	
1. Prepared with alcohol and ether.	56.84	56.67	56.76	4.45	4.36	4.41	1.96
2. hydrochloric acid.	56.67	56.35	56.51	4.04	3.96	4.00	0.28
3. glacial acetic acid.	56.26	—	56.26	4.26	—	4.26	0.28
4. basic lead acetate.	56.61	56.33	56.47	3.76	3.72	3.74	0.15
Mean value.			56.5			4.1	

It contains no reducing sugar, and yields by digesting with zinc-dust and water, gallic acid and phloroglucinol as observed in maletto tannin occurs in the bark of *Eucalyptus occidentalis*¹.

II. CONSTITUENTS OF SHIBUOL.

Studies of the well marked reactions of shibuol mentioned above, and of the colour reactions² of the hydrolytic products of the " tannin-masses " from the fully ripe fruit of the persimmon, by treating with

¹ F. Czapek; Biochem. Pflanzen. III, 498 (1921); A. G. Perkin and A. E. Everest: The natur. organ. colour. matters, 443.

² E. D. Clark; Loc. cit.

dilute mineral acids or alkali, led the authors to the conclusion that the chief constituents of shibuol, formed by hydrolysis, should be phloroglucinol and gallic acid.

Shibuol was, therefore, subjected to alkali-fusion to ascertain the above idea by isolating these compounds from the reaction products.

(1) FUSION WITH ALKALI.

5 grms. shibuol prepared by the second method, were digested in a silver dish with 15 gm. caustic potash and 5 c.c. water, until the mixture became pasty. The fused mass was dissolved in 200 c.c. water, and acidified with dilute sulphuric acid, whereas a brown amorphous precipitate (A) was formed, and filtered. The filtrate was evaporated to dryness (mass B) under reduced pressure.

The dark brown mass (B) was treated with a mixture of 140 c.c. 94 % alcohol and 210 c.c. ether in Sudo-Kumagawa's apparatus for 6 hours to extract phloroglucinol and gallic acid formed by hydrolysis.

The extract was subjected to distillation to get off the solvent, and the residue dissolved in 200 c.c. water. To separate gallic acid from phloroglucinol, the aqueous solution was treated with neutral lead acetate solution to precipitate gallic acid only as an insoluble lead salt (C), and filtered. The precipitate (C) was washed with water, suspended in water and decomposed by means of sulphuretted hydrogen.

The aqueous solution containing gallic acid, was separated from lead sulphide, and concentrated under reduced pressure, and gallic acid was crystallised in needle form thereby. It melted at 239-240°. The yield was 0.08 gm.

The filtrate containing soluble lead salt, separated from precipitate (C), was also treated with sulphuretted hydrogen gas to get phloroglucinol in a free state. The solution, separated from lead sulphide, was evaporated under reduced pressure. Phloroglucinol amounting to 0.2 gm., was obtained in a crystalline state. It melted at 209-211°.

Two specimens obtained in a pure crystalline state, were identified to be gallic acid and phloroglucinol respectively, by studying the colour reactions towards ferric chloride and also by determination of their melting points.

The substance supposed to be gallic acid was dried at 100° in vacuo for 5 hours, and was analysed with the results : C=49.36 ; 49.42; H=3.57 ; 3.65 (theory requires C=49.41 ; H=3.52 for $C_6H_2(OH)_3COOH$).

Phloroglucinol, obtained from shibuol, was analysed after being dried at 60° in vacuo for 5 hours : C=56.88 ; 56.34 ; H=4.50 ; 4.80 (theory requires C=57.14 ; H=4.76 for $C_6H_3(OH)_3$).

(2) PHLOROGILUCINOL-CONTENT IN SHIBUOL.

This was estimated approximately by following E. Vrotocěk and R. Potmešil's directions¹ which were used in the estimation of phloroglucinol in phloretin, apigenin etc.

Phloroglucinol formed by potash-fusion, was precipitated with furfuraldehyde in hydrochloric acid solution. It was expected the yield of furfuroolphloroglucide will be effected by the formation of an insoluble mass after the fused solution was acidified.

0.436 grm. Shibuol prepared by the third method, were digested with 7 grm. caustic potash and 2.5 c.c. water, acidified with 34.8 c.c. conc. hydrochloric acid and made up to 100 c.c. Phloroglucinol formed in the solution, was precipitated as furfuroolphloroglucide, which was filtered after 24 hours on Gooch's crucible, washed, and dried at 105° in an atmosphere of hydrogen gas.

The amount of phloroglucinol was calculated from the phloroglucide (weighing 0.3258 grm.), to be 49.7 %.

The phloroglucinol content in other samples, was also determined, and the results were as shown in the table II.

¹ Ber. D. Chem. Ges., **49**, 1185 (1916).

² Monat. Chem., **6**, 989 (1885).

(3) METHOXY VALUE OF SHIBUOL.

It was estimated by following the Zeisel direction² as usual and the results were as shown in the table II.

TABLE II.

Samples.	Methoxy Value.		Phlorogluciol.
1. Prepared with alcohol and ether.	0.18	0.23	49.12
2. „ „ hydrochloric acid.	0.85	—	49.71
3. „ „ glacial acetic acid.	0.27	—	42.86
4. „ „ basic lead acetate.	0.46	—	39.67

(4) THE INSOLUBLE SUBSTANCE (A)

It was a brown amorphous substance soluble in alkali. The carbon and hydrogen content in the sample and also the methoxy group were determined with the following results :

C.	61.70 ;	61.72	mean.	61.71
H.	3.10 ;	3.29	„	3.20
CH ₃ O.				1.57

It contains more carbon and less hydrogen as compared with shibuol.

A comparison of the ultimate analysis, indicates much resemblance with the brown substance named "goma," found in the pulp of ripe fruit of the sweet variety and also of the desiccated fruit.

III. KAKI-GOMA.

(1) ISOLATION OF KAKI-GOMA.

It was a brown amorphous substance formed naturally in the fruit of the sweet variety, and also found in the desiccated fruit of the astringent one¹.

The kaki-goma used in this investigation was isolated in the following ways from the pulp of the desiccated fruit named dojo-hatiya, produced at Ogaki, Gifuken.

¹ S. Komatsu and Ueda: Loc. cit.,

(a). The pulp was digested with water to remove soluble matter such as simple sugars and pectin and then treated with 72 % sulphuric acid to dissolve cellulose remaining with goma in the pulp, and washed again with boiling water.

20 gm. of crude goma thus prepared, were treated with 100 c.c. of 10 % acetic acid, washed with water, and shaken with 72 % sulphuric acid in a flask for 3 hours and finally washed with water to remove the acid completely. The yield of goma was 6.3 gm.

To purify the crude goma, 34 gm. of the sample, were treated with 300 c.c. hot alcohol, washed repeatedly with water, and dried.

The dried substance was sifted with a 50 mesh-sieve, and yielded 9.3 gm. pure goma.

(b). It was treated with 100 c.c. 72 % sulphuric acid for 5 hours, and the soluble matter washed off. Some impurities associated with the goma can easily be removed mechanically by decantation from the goma, which sinks to the bottom of the vessel. On the process being repeated, fairly pure goma was obtained. The yield was 8 gm.

It was soluble in alkali, but insoluble in all organic solvents, and gave the following results on analysis after being dried at 130° for 3 hours :

C.	59.90 ;	59.83	mean	59.87
H.	3.84 ;	3.67	,,	3.76
CH ₃ O.				1.94
Phloroglucinol.				17.88

(2) POTASH FUSION OF KAKIGOMA.

6.3 gm. kaki-goma were digested with 20 gm. caustic potash and 7 c.c. water in a silver dish. The fused mass was dissolved in 150 c.c. of water, and acidified with sulphuric acid, whereupon a dark brown precipitate was formed, which was separated by filtration, and washed with hot water.

The combined solution of filtrate and washings, was evaporated as usual under reduced pressure, and the phenolic compounds extracted with a mixture of alcohol and ether. The residue remaining after the solvent had been evaporated off, was dissolved in water, and treated with neutral lead acetate.

The insoluble lead salt, thus formed, was confirmed to be a salt of gallic acid, the free acid being isolated by the usual processes (m. p. 239—240°). The yield was 0.15 gm.

From the filtrate containing soluble lead salt, however, a trace of a substance, was obtained giving the same colour reaction as phloroglucinol towards ferric chloride.

In the same manner, using 3.5 gm. pure goma, we obtained after hydrolysis 1.8 gm. of a dark brown substance, insoluble in an acidic solution.

It was dried at 130° in an air-bath for 3 hours, and analysed with the following results :

C.	62.99 ;	62.78 ;	mean	62.88
H.	3.74 ;	3.75 ;	,,	3.74

It is anticipated that the transformation of shibuol by hydrolysis into a brown substance will be shown to have some connection with the formation of kakigoma in the fruit, in course of ripening, and the relation between kaki-goma and phlobaphene will be discussed in a succeeding article.

Summary.

1. The shibuol isolated by the precipitation methods, on analysis gave numbers expressed by the empirical formula $C_{14} H_{20} O_9$.
2. Shibuol, on fusion with potash, gave a substance represented by the formula, $C_{12} H_8 O_5$, and phloroglucinol and gallic acid.
3. Kakigoma of the empirical formula, $C_{11} H_9 O_5$, by potash fusion, yielded some phloroglucinol and a large yield of gallic acid and a substance represented by the formula, $C_{13} H_9 O_5$.