CHEMICAL STUDIES ON MENTHA*

I. On the Chemical Compositions in the Plant of the "San-Bi" (Mentha Arvensis, L.) and the Transmuted "Aka-Kuki" **

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長沢 徹・曾我 治： 薄荷属植物の化学的研究
第1報 「三美」及び変異「赤茎」種の化学成分研究

I. INTRODUCTION

The production of mint[2], which has been stood on the important position as the Japanese useful agricultural harvest, is greater in Hokkaido then followed by Okayama prefecture that is San-Bi districts[1]. But in view of the point of quality the latter is superior to the former[3]. Both species belong botanically to the *Mentha arvensis*, L. (Japanese Mentha). After drying the harvested mint grasses the "Torioroshi-Oil" (Natural Oil) was manufactured by steam distillation and separated to the Menthol Crystals and the Dementholized Oil. Both of which are purified as an article for export and the demands in the land.

In Hokkaido, where harvested in large scale, the cultivating area of mentha attained maximum over 20 thousands Cho, and produced 1,300 thousands Kin of Torioroshi-Oil in the year 1937. However, we had about one thousands Cho of the area and 130 thousands Kin of Torioroshi-Oil in Okayama prefecture on the same year. Contrary to the Okayama district, which has thrice harvest times (June, August, and October), Hokkaido has only the once crop (September). That shows the climate of Okayama area gave a very good

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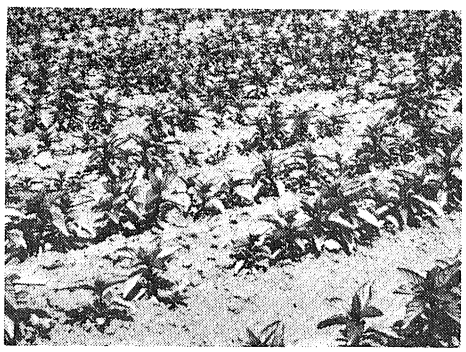
* The outlines of this paper have been published as "Chemical Studies on Mint Plant", I and II separately at the 17th meeting (at Matsue, July, 1955) of the Chugoku-Shikoku Branch of the Chemical Society of Japan and at the 9th annual meeting (at Kyoto, April, 1956) of the same society, respectively.

** "San-Bi" is the finest mint species (*Mentha arvensis*, L.) now cultivated in Okayama prefecture.

Transmuted "Aka-Kuki" is the worse mint ever cultivated in the suburbs of Matsue city.

conditions for the cultivation of mint. As the yields of the natural oils of Mentha upon their dry herbs are 1～2%, but the amounts of the oil production in one Tan both in Hokkaido and San-Bi district are 5 and 15～20 Kin, respectively.



(May 6, 1956)

(Aug. 13, 1956)

Photograph of the "San-Bi" (*M. arvensis*, L.)

Since the Menthol Crystals and the Dementholized Oil have an uncomparable cooling taste and fragrance, they are used in the dental agent on every day life as the necessary materials. And they are used widely as in candies, liqueur, cooling drinks, chewingum, tobacco, medicines (especially stomatics), mentholatum etc. The Japanese peppermint had hitherto satisfied almost the demands of the world, the Chinese mint[4] was out of thinking, but the natural mint in Brazil has appeared[5] after the War II with steady and fundamental state. At the present, therefore, the main producing countries of the natural mint are Japan, Brazil, and China, which became to a state of competition in each other. Also the countries which have not produce the natural mint have now manufactured commercially *synthetic menthol* and consequently the *synthetic menthol* has come to stand against *natural menthol.*

It is important generally to increase the production of the Japanese mint to go abroad with a great ambition, but rather demanded to produce menthol crystals and peppermint oils with superior qualities. One of the authors, T. Nagasawa [2,20,21,22,23,25,27,28,29,34], during five years (1949～1953) of his stay in Okayama prefecture, thought severely that the most important counter-measure is to cultivate the good species of Mentha and has studied on the progress and breeding of the superior Japanese mentha in cooperation with

E. Nasu[6], a special engineer of Okayama prefecture, both H. Inoue[41,42,44] and S. Chamura [48,49,50,51], engineers of the Okayama Agricultural Experiment Station, and Prof. N. Ikeda [7,45,46,47] of Okayama University. At last we have percieved and excluded the worse mint (as Dog-mint and Horse-mint)[20,21,22,34] which spread over the field after the War, and we have found "Beni-Hakka" (Crimson-Mentha)[24,25,26,27,28] of the finest species that has ever seen before, and we named it the "San-Bi"[29,30] (1953). The species "San-Bi" has excellent points as much yield in production[24,25,26] of herbs and oils, stout to sickness, and the highest content of *crystal menthol* without bitter taste[14,27,28].

The mentha species hitherto have been cultivated in Okayama prefecture are"Aka-Kuki" (Red-Stem), "Ao-Kuki" (Green-Stem), and "Shiro-Hana" (White-Flower), but now the "San-Bi" (Three-Beauty) has came as the representing species with superior qualities, it was cultivated in 1954 as in the TABLE 1. And it was interchanged to spread over 80% of the mentha of 1,000 Cho in Okayama prefecture.

TABLE 1. Cultivation Area of Okayama-Mint(1954)[30]

Species	Aka-Kuki	San-Bi *	Ao-Kuki	Others	Total
** Area(cho)	359.83	69.33	42.16	34.46	505.78
Ratio(%)	71	14	8	7	100

* San-Bi in Okayama prefecture has been expanded to above 80% of the whole area of mentha this year(1956).

** 1 cho=2.45 acre.

On the constituents of the Japanese mint oils, Beckett and Wright (1876)[8] have found originally *l-menthol* as a crystal matter, Moriya(1881)[9], then, detected *menthone*. After them, Murayama (1910)[10] found *l-limonene* so the main constituents of the mint oil seem to become known already. Moreover, Shinosaki- Nagasawa[11,12], and Tanaka[13] investigated simultaneously the mint oils in details. Hence the studies on the utilization of mint oils by Shinosaki-Nagasawa[16], Hayashi[17], and Ito[18,19] are expanded for the contribution to establish the Japanese peppermint industry.

Looking again for the Japanese Mentha, every mint species were bred each other severely in order to wilderness by defeat of the War, we have found "Uma-Hakka" (Horse-mint) [22] besides "Inu-Hakka" (Dog-mint)[6,21] as a bad species and studied the methods of rapid distinguishment of mint species[21,25], and Nagasawa percieved the relative viscosity method [22,23] and color test[24] as invaluable for the diagnosis of the natural oil. Here we would cite the data of newly found "San-Bi" by Nagasawa[27,29] and Chamura[24,26] in the following tables (TABLE 2~6).

TABLE 2. Growth of Mint Species and the Oil Yields (1952)[29]

Time of Crop (Year, Month)	Oil Yields (upon Dry Herbs, %)			
	San-Bi	Aka-Kuki	Ao-Kuki	Shiro-Hana
1952.6	0.76	0.74	0.47	0.73
* 1952.8	1.58	1.49	1.12	1.66
1952.10	1.34	1.41	1.00	1.39

* Max. oil yield on August.

TABLE 3. Relation of Growth of Mint and Menthol-Content, in the Oil[29]

Time of Crop (Year, Month)	Free Menthol (%)			
	San-Bi	Aka-Kuki	Ao-Kuki	Shiro-Hana
1952.6	81.3	74.9	76.5	73.7
1952.8	83.6	77.2	77.6	78.3
* 1952.10	86.1	80.8	78.7	80.3

* Menthol contents in the Torioroshi-Oil (Original Oil) increase with maturity.

TABLE 4. Properties of Every Mint Oils (2nd Crop)[27]

Species	Oil Yieds (to Dry Herbs) (%)	d_4^{25}	n_D^{25}	η^{25}	* EM (%)	** FM (%)	Crystal-Menthol (%)
!! San-Bi	1.6	0.896	1.461	17.4	2.2	83.6	64
Aka-Kuki	1.4~2.4	0.895~897	1.460~1.461	9.9~13.3	3.0~7.1	73.2~79.4	49~59
Ao-Kuki	0.7~1.7	0.895~889	1.460~1.462	10.6~13.3	3.4~6.6	75.8~80.6	50~58
Shiro-Hana	1.6~2.4	0.895~896	1.460~1.461	11.2~13.1	3.6~6.6	77.3~79.6	54~59

* EM=Ester menthol.

** FM=Free menthol.

!! San-Bi has the highest content of Crystal-Menthol and also the highest value in Relative viscosity.

TABLE 5. Comparison of Every mint Products in One Tan[29]***

(Cultivated at Kurashiki-Bunjo)

Species	Fresh Herb (Kan) !	Torioroshi-Oil(Kin)!!	Average Crystal-Mentbol (%)	Crops of Crystal-Menthol (Kin)	Calcd. to Standard Oil(Kin)	Ratio **
* San-Bi	785	15.5	65	10.0	17·8	144
Aka-Kuki	512	11.7	56	6.5	12.4	100
Ao-Kuki	658	10.3	52	5.4	10.5	85
Shiro-Hana	450	11.0	57	6.3	11.8	95
Hokushin	530	8.4	56	4.7	8.9	72

* Indicates the San-Bi is the best species, which produces 25 Kin of Torioroshi Oil (original oil) under good conditions.

** Aka-Kuki is the standard Mint hitherto in Japan.

*** 1Tan = 0.245 acre

! 1Kan = 3.75 Kg

!! 1Kin = 0.6 Kg

TABLE 6. Examination at Kurashiki-Bunjô (4 Tsubo-Cultivation)** [24]

Species	Fresh Herb ! (Monme)	Dry Herb (Monme)	Torioroshi Oil (Monme)	Oil Yield (%)	Average cryst. Menthol (%)	(Dec.) Root-Stocks (Monme)
*San-Bi	10,008	2,430	33.0	1.35	65	1,730
Aka-Kuki	6,541	1,782	22.3	1.25	57	338

* The San-Bi has very much yield compared with Aka-Kuki, especially the former has five times more in root-stocks than the latter on December.

** 1Tsubo = 0.000816 acre

! 1Monme = 3.75 g

The constants of the essential oil of "San-Bi", by Nagasawa's report[29], are as follows:-

d_4^{25}	0.8956
n_D^{25}	1.4612
η^{25}	17.4
$[\alpha]_D$	−43.4°
F.P.	20.2°C
Ester Menthol	2.2%
Free Menthol	83.6%
Menthone	5.0%
Unsaturated *Ketone*	1.4%

Shimizu[33] has investigated recently micro quantitative analysis of mint oils by the polarographic method which seemed to be promising.

On the other hand, in Hokkaido, the main district of northern region of Japan, the Mentha has been improved by many researchers long before. Kitamura[31] and others have endevoured to find good species that "Aka-Maru"→"Kitami-Shiroke"→ "Hoku-Shin" (1938), and Kasano et al. [32,43] have found recently(1952) a good species, which named "Man-Yo", of rich oil yields by the artificial breeding. Oil yields of them are in the TABLE 7.

TABLE 7. Mint Species in Hokkaido[31,32]

Species	Yield in Tan		In Torioroshi Oil	
	Fresh Herb (kan)	Torioroshi Oil (kan)	Crystal Menthol (%)	FM (%)
Aka-Maru	422	0.763	46	72.9
Hoku-Shin	655	1.209	59	80.3
* Man-Yo	951	2.100	62	78.8

* Man-yo has two times of yield over Aka-Maru, and produced 17.5 Kin of Torioroshi-Oil which contained above 60% Crystal-Mehthol.
Thus we see the great development of mint in Hokkaido.

We have commenced this research as one corner of the studies on the progress of Japanese Genus Mentha[29], and for investigating the chemical change of the plant substances on relation to the formation of the essential oils during the growth of Mentha. These chemical studies, therefore, would be continued hereafter. We investigated in this report the chemical change in each plant position at one season (3rd. crop) of two species of Mentha.

Ⅱ. GENERAL REMARKS ON THE RESEARCH

Mentha germinates from the root-stocks early in the spring and passing the maximum period of the summer (August) to wither before the fall of the frost. To study the seasonal biological change of mint grass, we took five portions equally divided along with the length of the stem.

As the growth of mint finishes in one year, we made attempt to compare the rapid growth of the bamboo. One of the writers (Nagasawa)[36] has ever investigated the biochemical studies of the bamboo with a guidance of late Dr. Prof. Komatsu. We have known the following fact in the bamboo that the total *ash*, *nitrogen*, and alcohol-soluble substances were increased similarly to the upper positions from the ground as 1.42→2.59%, 0.26→0.60%, and 4.09→8.80%, respectively. The total *reducing sugars* and *cellulose*, on the contrary, were decreased along with the hight as 2.37→1.17%; and 54.3→50.6%, respectively. Tanaka[3] had reported in his study of bamboo shoot the same results that both *ash* and *nitrogen* were increased to the top as 7.32 →12.88%; and 2.72→5.78%, respectively, and the total *reducing sugars* and *cellulose* were decreased inversely as 16.6→9.9%; and 29.6→8.5%, respectively. In other words, it has confirmed that *ash* and *proteins* were formed much in the upper young portions of rapid growing bamboo

but *sugars* were stocked rich in the lower matured part.

We have obtained the similar results in this research of mentha. The chief engineer Fukushima[37,39] in Kurashiki Branch of Okayama Agricultural Experiment Station has investigated on the essential oil related to the growth of mint, by the suggestion of Nagasawa, with micro-distillation wich brought very interesting results as in the TABLE 8. Thus we see the maximum oil yield in the upper youngest developed leaf, and decreasing to the lower old leaf. This shows that the formation of the essential oil[15] be commenced even at the young shoots.

TABLE 8. Oil yields in the Leaf position of Aka-Maru (8/Ⅷ)[38]
(Oil contents in the Leaf)

* Leaf position		Oil yields (%)	
		Upon Fresh Leaf	Upon Dry Leaf
Upper	(1)	1.68	7.18
	(2)	1.72	8.27
	(3)	1.37	5.26
	(4)	1.20	4.37
Lower	(5)	0.61	2.20

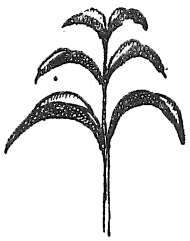
* (1) is the top of the stem and the young leaves are not yet developed.
(2) is the first pair of the developed leaves.

Miyake and Ishizuka[39,40], on the other hand, harvested the "Kitami-Akamaru" to measure the growth of each in the intervals of 10 days. They have improved that the weight of fresh herbs, mint oil, and *menthol crystals*, and oil yield are all increasing with growth, attaining to the maximum at full bloom and then to the lowering of the yield with the lapse of mature.

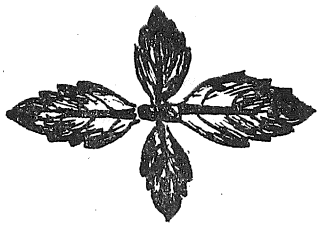
Samples

We studied the chemical compositions in each position of the plants to investigate the biochemical change of the mentha. The samples used in this research are (A) "San-Bi" whose rootstocks were presented from Okayama Agricultural Experiment Station and cultivated at Matsue city, and(B)"Interchanged Aka-Kuki" (we shall call it "Aka-Hen" hereafter) which had been transported here few years ago from Okayama prefecture.

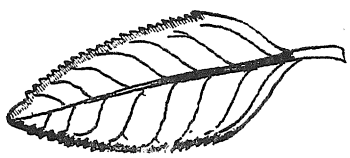
The "San-Bi" has a remarkable character of generating the prominent purple colors of anthocyane along with the young leaves on the month of May(FIG. 1.)[28].



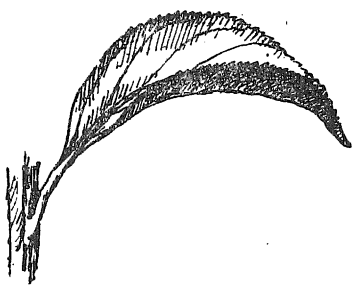
(d) General form of a Plant
in the Upper Part
(Sept.11th,1953)



(a) Young Leaves at the Top
(May 15th,1953)



(b) Second Leaf from the Top
(Aug.3rd,1953)



(c) Bending Form of a Leaf
(Sept.11th,1953)

(e) Botanical Form
(Sept.11th ,1953)

FIG.1. Emergence of Pigments and Botanical Forms of "San-Bi" (1953) *

* From the *Koryo*, No. **29**,18~30 (1954)
(Sketched by Nagasawa.)

Also the stem is colored purple and the leaves are generally dark green which can be seen from fair distance. The "Aka-Hen", on the other hand, has a pale violet green stem with pale green leaves. A stem of the "San-Bi" is, in general, thick and stout but that of "Aka-Hen" is thin and tends to extend rapidly. We can scent strongly the fragrance of *menthol* from the former, but weak and queer odor in the latter. The attaching of the leaves is more compact in the former than the latter.

We cut the both species of mentha (A and B) at 10~11 o'clock on Oct. 20th, 1954 (3rd crop). After weighing the fresh herbs we divided them to five equal portions along with their length from bottom to the top (I→V) and separated the leaves from each stem. The states of their growing are showed in the TABLE 9 and the FIG. 2.

TABLE 9. State of Mint in this Research (Oct. 20, 1954)
Fresh Herbs (20 Stems)

Species	Average Length (cm.)	Av.no. Leaf-Knots	Total weight (g.)	Leaf wt. (g.)	Stem wt. (g.)	Ratio Leaf: Stem	Av. Single Weight (g.)
* San-Bi	32.8	11.0	143	90	53	1.7:1	7.2
Aka-Hen	22.7	10.0	128	83	45	1.8:1	6.4

* San-Bi is in better states of growth than Aka-Hen.

TABLE 10. Essential Oil Yield in Leaf-position. (upon Dry Leaf)

Position		San-Bi (%)	Aka-Hen (%)
(Lower)	I	—	—
	II	—	—
	III	2.88	0.45
	IIII	3.15	0.90
(Upper)	V	3.83	1.13
	Mean	3.29	0.83

N.B. The oil yield is rich in upper leaves.
San-Bi has the oil yield of four times to Aka-Hen.

As the results of the microdistillations in TABLE 10, we percieved the oil yield of the "San-Bi" is greater four times that of the "Aka-Hen" and both the upper leaves have a higher oil yield.

Comparing A(San-Bi) with B (Aka-Hen), both cultivated experimentally in the field of the Shimane University (1956), we have the following results (TABLE 11, 12).

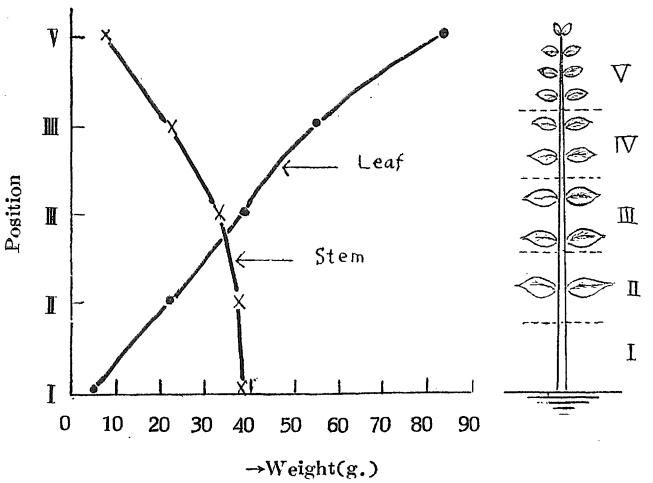


FIG.2. Weights of leaf and stem at each position of "San-Bi" (Dry weights of 270 herbs)

TABLE 11. Properties of Mint Oil from San-Bi (Cultivated at Shimane Univ., 1956)

Time of Crop	Oil yield (%)		d_4^{25}	n_D^{25}	η^{25}	$[\alpha]_D^{10}$	EM (%)	FM (%)
	On Fresh Herb	On Dry Herb						
1956.Ⅷ.20(Ⅰ)	0.57	2.27	0.8942	1.4626	14.46	-40.3°	1.63	79.65
1956.Ⅹ.22(Ⅲ)	0.33	1.37	0.8944	1.4623	14.47	-42.9°	0.80	81.29

EM = Ester Menthol

FM = Free Menthol

TABLE 12. Properties of Mint Oil from Aka-Hen (Cultivated at Shimane Univ., 1956)

Time of Crop	Oil Yield (%)		d_4^{25}	n_D^{25}	η^{25}	$[\alpha]_D^{15}$
	On Fresh Herb	On Dry Herb				
1956.Ⅵ.4 (Ⅰ)	0.03	0.22	—	1.4862	—	-17.4°
*1956.Ⅷ.20(Ⅱ)	0.08	0.26	0.9139	1.4869	2.42	-13.7°
1956.Ⅹ.22 (Ⅲ)	0.06	0.24	0.8957	1.4842	—	-30.0°

* The Oil of Ⅱ was analyzed[27] as follows: Ester Menthol 7.96%; Free Menthol 16.24%.

Judging from these data, as (1) lower oil yield, (2) larger in refractive index (n), (3) lower in relative viscosity (η), and (4) much less in free menthol, we think this rank, Aka-Hen, to be quite abnormal ones. We regard it has transformed to bad rank. Thus we have found the retarding of Mentha in Shimane Prefecture.

We confirmed that A showed good results (oil yield, 2% ; *free menthol*, 80%) of the "San-Bi" as in Okayama and B, on the contrary, was inferior (oil yield, 0.2%; *free menthol*, 16%) to Dog-mint nearly.

Summary of the Research

We have acknowledged in this research a remarkable differences in the chemical components between the "San-Bi", good species, and "Aka-Hen", worse species. The "San-Bi" contained much more quantities of essential oils and *menthol crystals* than those of the "Aka-Hen". The *nitrogenous compounds* are particularly much in the former (TABLE 16), but the carbohydrates contained greater in the latter (TABLE 15), that indicates the deep relation with the formation of essential oils. Also it may be attentive that the "San-Bi" absorbed much quantities of the nutrition (TABLE 13, 14).

TABLE 13. Contents of Inorganic Substances (upon water-free basis, %)

Average Contents	San-Bi		Aka-Hen	
	Leaf	Stem	Leaf	Stem
Total ash	14.37	10.56	14.04	9.10
P_2O_5	1.68	0.79	2.04	1.21
K_2O	4.49	3.09	4.22	2.73
CaO	5.33	2.10	5.04	0.33
MgO	0.31	0.45	0.27	0.33
SO_3	2.32	0.82	2.44	1.22
SiO_2	0.98	0.26	0.90	0.17

TABLE 14. Contents of Organic Substances (upon water-free basis, %)

Average Contents	San-Bi		Aka-Hen	
	Leaf	Stem	Leaf	Stem
Carbohydrate	9.62	26.11	14.70	33.23
Protein	23.08	7.78	17.24	5.60
Fat	6.13	1.21	5.40	0.91
Cellulose	15.5	27.3	12.9	24.2

We summarized our preliminary studies as follows : -

1) Judging from larger content of *ash* in the "San-Bi" (14.4%) than in the "Aka-Hen" (14%), we see much absorption of the nutritive elements in the former. And it is supposed to increase the quantity of the essential oils in the herb by catching nutrition

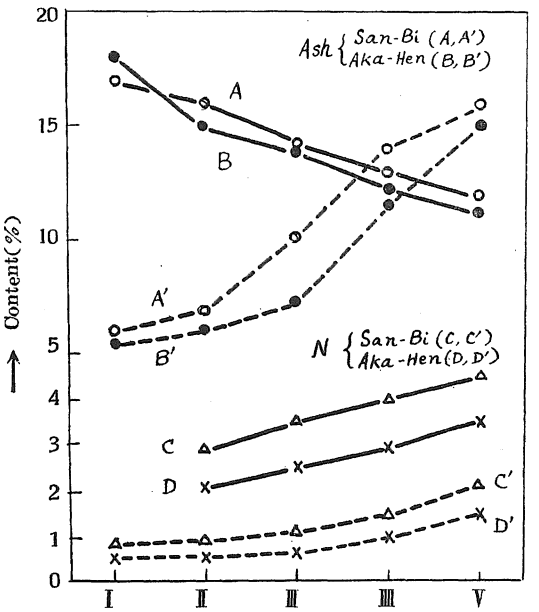


FIG.3. Total Nitrogen and Ash in "San-Bi" and "Aka-Hen"
(Full lines: Leaf; Broken lines: Stem)

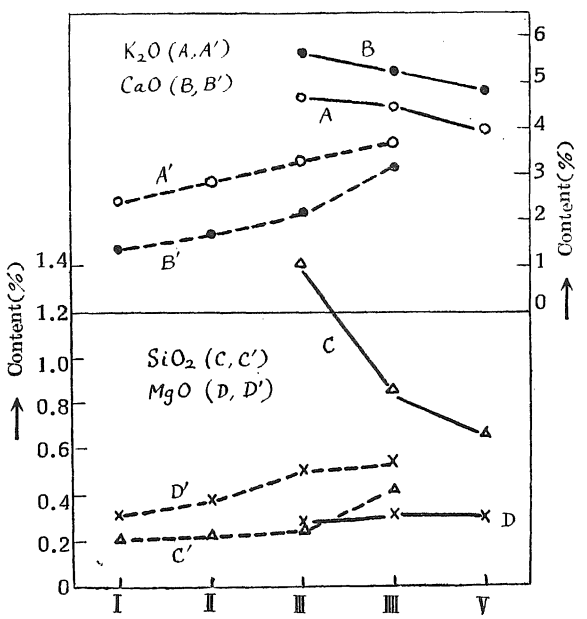


FIG.4. Ash content in "San-Bi"
(K_2O, CaO, SiO_2, MgO)

(TABLE 11, 12, 13; FIG. 3).

2) The fact that the *ash* contained much (15.2%) in the upper position of the stem means the necessity of much nutritives for the sake of the lively split of cells at the tip of the stem. To have a larger quantity of *ash* (16.6%) in the lower leaves than the upper ones seems to deposit the nutritives in the matured leaves. Especially the influence of SiO_2, CaO, and K_2O are great (FIG. 4).

3) It is said that the *sugars* and *starch* are increased with an addition of *phosphoric acid*, but *proteins* are decreasing. Comparing the "San-Bi", rich in *nitrogen* (3.6%) and *proteins* (23%), with the "Aka-Hen", wealthy in *sugars* (14.7%) and *phosphoric acid* (2.0%), we recognized very interesting fact in the chemistry of plant life. It is delightful that we know the acidic oxides as P_2O_5 and SO_3 are contained more in the "Aka- Hen" (2.0; 2.4 %) than "San-Bi", but the alkaline oxides (K_2O, 4.5; CaO, 5.3%) are rich in the latter (TABLE 13,14; FIG. 5).

4) Viewing to the state of sbsorption of the nourishment we can divide it three groups of K_2O - CaO ; N-P_2O_5-SO_3; and MgO-SiO_2 similar to riceplant.

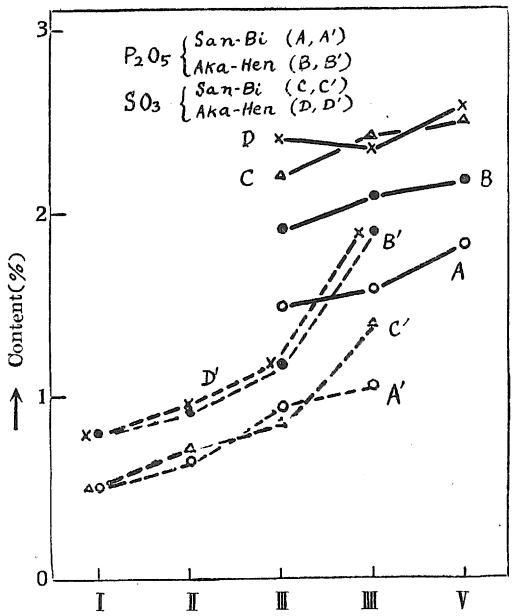


FIG.5. Ash content in "San-Bi" and "Aka-Hen" (P_2O_5, SO_3)

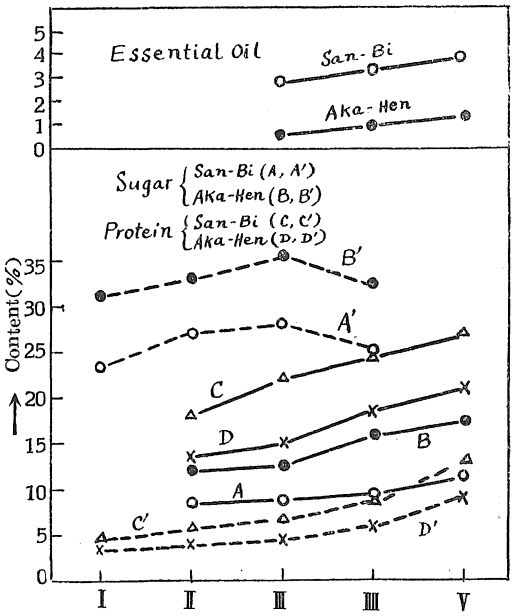


FIG.6. Sugar, Protein, and Essential Oil in "San-Bi" and "Aka-Hen"

5) The order of the each content in the plant was nearly as the following:-

$CaO > N > K_2O > SO_3 > P_2O_5 > SiO_2 > MgO$ (TABLE 13,16).

6) The contents of the essential oils are abundant in the upper leaves, the "San-Bi" was above the twice the "Aka-Hen" (TABLE 10,11,12; FIG. 6).

7) The *carbohydrates* contained more in the "Aka-Hen" (14.7%) than the "San-Bi" (9.6%), which predominate in the upper leaves (11.4%) and middle stems (28.1%). The *reducing sugars* are contained rich in the upper positions (5.7; 6.8%) of both leaves and stems. *Non-reducing sugars* of the leaf are much somewhat in the young part (1.4%), and those of the stem are abundant in the middle part (17.5%). *Starch* contained much in the middle part of both leaves and stems (5.4; 8.0%) of which more in the stems than the leaves. Both *reducing sugars* and *non-reducing sugars* exist more in the stems than the leaves (TABLE 15; FIG. 7).

TABLE 15. Contents in Carbohydrates (upon water-free basis, %)

Average Contents	San-Bi		Aka-Hen	
	Leaf	Stem	Leaf	Stem
Total Sugar	9.61	26.11	14.70	33.23
Soluble Sugar	4.95	20.40	10.71	25.41
Reducing Sugar	3.86	5.81	7.96	9.22
Non-red. Sugar	1.10	14.60	2.76	16.19
Starch	4.66	5.78	3.99	7.82

8) The *nitrogenous compounds*, contrary to *carbohydrates*, contained rich in the "San-Bi" (3.6%) (TABLE 16). Both the *proteinous* and *soluble nitrogen* predominated at the point of growth (upper parts) especially in the leaf (3.3; 1.1%) (TABLE 21; FIG. 8).

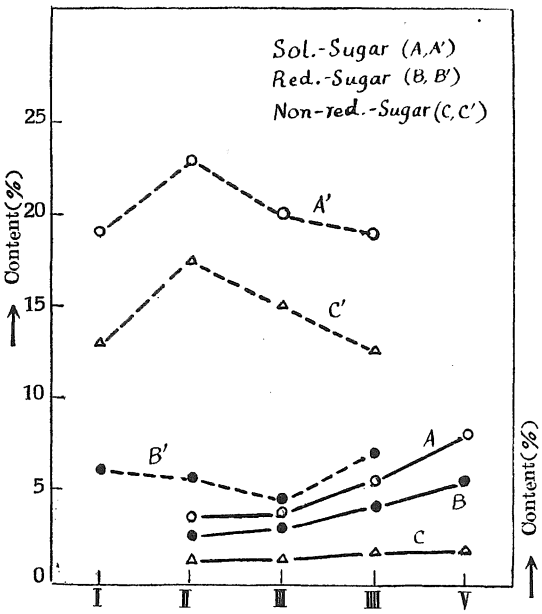


FIG.7. Sugar content in "San-Bi"

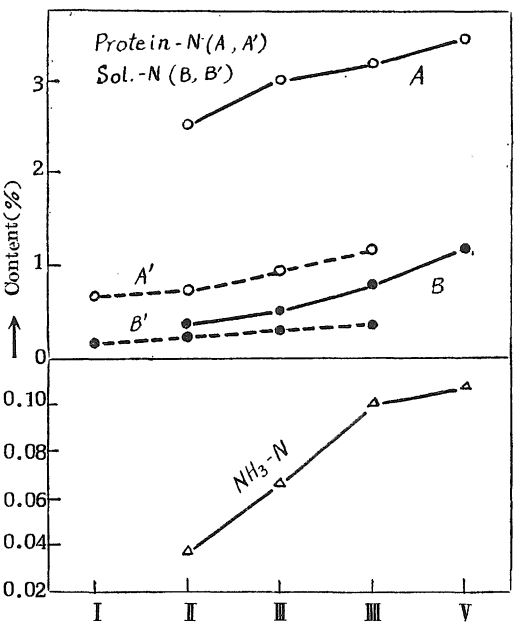


FIG.8. Nitrogen content in "San-Bi"

TABLE 16. Contents in Nitrogen (upon water-free basis, %)

Average Contents	San-Bi		Aka-Hen	
	Leaf	Stem	Leaf	Stem
Total-N	3.66	1.24	2.76	0.89
Protein-N	3.00	0.81	2.39	0.56
Soluble-N	0.69	0.22	0.36	0.19
NH_3-N	0.08	—	0.05	—

9) The *fat* is abundant in the lower leaves (7.1%) and in the upper stems (1.7%), and more in the leaf (6.1%) than the stem (1.2%) (TABLE 14; FIG. 9).

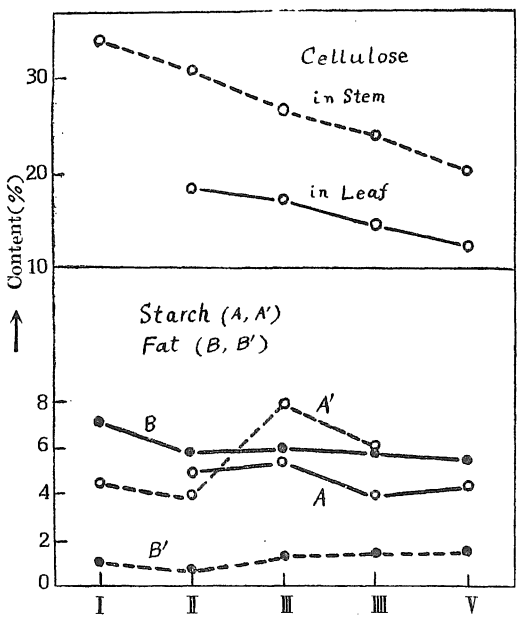


FIG.9. Starch, Fat, and Cellulose in "San-Bi"

10) The *cellulose* is defficient in the upper younger leaf and stem (12; 19 %) of the plant increasing with the growth (18; 35%) (FIG. 9).

11) In general, the *proteins* (23%) predominated in the leaves but the *carbohydrates* (26%) were rich in the stems (TABLE 14; FIG. 6).

III. EXPERIMENTAL PART

(1) **Sample:** The divided leaves and stems are dried and powdered to 1 mm-mesh for the analyses (TABLE 9;FIG.2).

(2) **Analyses of Inorganic Components:** The inorganic analyses were mainly referred to Okuda's method[52].

1. **Ash:** About 2 g. of the sample was taken to the porcelain crucible and heated with a gas burner to carbonize it, then ignited in the electric muffled furnace at 500°C for 5 hours to the constant weight.

2. **Components of ashes:** SiO_2 in *ash* was separated at first as usual, and the filtrate and the washings are gathered to a 100cc.-solution from which we took each certain volume for measure the following components:. P_2O_5 was analyzed from 2 cc. of the solution with a volumetric method of *molybdenic acid*, 2 cc. with *potassium permanganate* volumetric method for CaO, 10 cc. with Hexyl gravimetric method for K_2O, 10~20cc. with *phosphoric acid* gravimetric method excluding *calcium* for MgO, and 5~10cc. with *barium chromate* color method for SO_3. These results are shown in the following TABLES 17 and 18.

TABLE 17. Contents of Ashes (A)—San-Bi (On Dry Basis, %)

Position		Total Ash	P_2O_5	K_2O	CaO	MgO	SO_3	SiO_2
Leaf	I	16.46	—	—	—	—	—	—
	II	15.62	—	—	—	—	—	—
	III	14.51	1.53	4.70	5.72	0.28	2.14	1.41
	IIII	13.51	1.59	4.51	5.37	0.34	2.36	0.89
	V	11.60	1.92	3.97	4.90	0.30	2.47	0.64
Stem	I	6.21	0.57	2.43	1.28	0.32	0.25	0.20
	II	7.69	0.68	2.85	1.73	0.39	0.73	0.20
	III	10.02	0.92	3.30	2.18	0.51	0.80	0.24
	IIII	13.70	1.01	3.79	3.19	0.57	1.48	0.41
	V	15.22	—	—	—	—	—	—

TABLE 18. Contents of Ashes (B)—Aka-Hen (On dry basis, %)

Position		Total ash	P_2O_5	K_2O	CaO	MgO	SO_3	SiO_2
Leaf	I	17.03	—	—	—	—	—	—
	II	14.93	—	—	—	—	—	—
	III	13.82	1.94	4.39	5.19	0.30	2.46	1.35
	IIII	13.11	2.05	4.34	5.62	0.31	2.34	0.82
	V	11.33	2.14	3.93	4.32	0.20	2.51	0.53
Stem	I	5.69	0.80	2.43	0.24	0.24	0.79	0.13
	II	5.72	0.93	2.27	0.26	0.26	0.95	0.14
	III	7.41	1.22	2.71	0.34	0.34	1.24	0.18
	IIII	11.53	1.91	3.50	0.48	0.48	1.90	0.22
	V	15.12	—	—	—	—	—	—

(3) **Analyses of Organic Components**[53] :

1. **Carbohydrates**: The sample of 0.5 g. with 50cc. of water and 2cc. of 30%-*sulfuric acid* were hydrolyzed 2 hours on the water bath. After the hydrolysis it was neutralyzed with 30%-*sodium hydroxide* solution, filtered and washed to the 100 cc-solution from which we took 20 cc. for measuring the *reducing sugars* (as *glucose*) with Bertrand's method.

Chemical reactions in Bertrand method:-

$$RCHO + 2Cu(OH)_2 = Cu_2O + RCO_2H + 2H_2O \quad \cdots\cdots (i)$$

$$Cu_2O + Fe_2(SO_4)_3 + H_2SO_4 = 2CuSO_4 + 2FeSO_4 + H_2O \quad \cdots\cdots (ii)$$

$$10FeSO_4 + 2KMnO_4 + 8H_2SO_4 = 5Fe_2(SO_4)_3 + 2MnSO_4 + K_2SO_4 + 8H_2O \quad \cdots\cdots (iii)$$

$$2KMnO_4 + 5H_2C_2O_4 + 3H_2SO_4 = 10CO_2 + 2MnSO_4 + K_2SO_4 + 8H_2O \quad \cdots\cdots (iv)$$

Reagents:- (a) **Copper Sulfate Solution.** 40 g.of pure *copper sulfate* ($CuSO_4.5H_2O$) was dissolved in distilled water and made up to one l. solution.

(b) **Alkaline Rochelle Solution.** 200 g.of *Kalium sodium tartrate* and 150 g. of *sodium hydroxide* were dissolved in water to one l. solution.

(c) **Ferric Sulfate Solution.** 50 g.of *ferric sulfate* and 200 g. of concentrated *sulfuric acid* were dissolved in water to one l. solution.

(d) **Potassium Permanganate Solution.** The one liter solution of 5 g.of *potassium permanganate* was stood for 2 or 7 days and filtered through glass filter "17G3", then preserved in the colored bottle. The concentration of $KMnO_4$ was determined with *oxalic acid* (equation (iv)). That is, one mole of the *oxalic acid* corresponds to $2Cu$. The quantity of *Cu* (mg.) corresponding to 1 cc. of $KMnO_4$-solution is, therefore, obtained from the following formula (v).

$$Cu(mg.) = \frac{\text{Weight of oxalic acid taken (g.)}}{\text{Titration volume of } KMnO_4\text{-sol. (cc.)}} \times \frac{2Cu}{H_2C_2O_4.2H_2O} \quad \cdots\cdots\cdots\cdots\cdots\cdots(v)$$

[(N.B.) 1 cc. of $KMnO_4$-solution corresponds to 10 mg. of *Cu*]

Manipulation of Analysis:- To the 200 cc.-Erlenmeyer flask(A) poured 20 cc. of the *sugar* solution (which contains 20~80 mg.of *reducing sugars*) with a pipette, and added again each 20 cc. of *copper sulfate* solution (a) and *Rochelle salt* solution (b). Then heated on wire gauze to boiling gently for 3 minutes and decanted to the glass filter "15AG4" attached to the Witt's filter bottle by slow suction. Again washed the flask (A) with 50 cc. of hot water and decanted as above. After repeating the decantation the receiver was changed with the former flask(A). Then poured 20cc. of *ferric sulfate* solution (c) into the filter at 3 or 4 times to dissolve the precipitates of *cuprous oxide* and filtered, and washed completely again into the flask (A) with a little hot water for several times. The filtered solution in the flask was titrated to the pink color with *potassium permanganate* solution (d) after the shaking.

If x(mg.) be the quantity of *Cu* in 20cc. of the *sugar solution*,

then we have $x = a.b$ (mg.)

where, a : quantity of *Cu*(mg.) per 1cc. of $KMnO_4$-solution.

b : titration no.(cc.) of $KMnO_4$-solution.

We can find the quantity of *glucose* (y mg.), corresponding to x mg. of *Cu*, using Bertrand's table, thus the *sugar* in the sample (100cc.) to be 5y (mg.).

2. Reducing sugar and Non-reducing sugar : The *reducing sugar* in the solution for the *soluble nitrogen* (see after) was analyzed as above, and, on the other hand, the *total soluble sugars* were obtained from 10cc. of the filtrate after the hydrolysis on the water bath with 2cc. of 30%-*sulfuric acid* for 2 hours. The quantity of the *non-reducing sugar* was calculated by the difference from the *total soluble sugars* to the *soluble reducing sugar*.

3. Starch : The *starch* was calculated as *glucose* by the difference from the *total sugars* to the *soluble sugars*. These results are shown in the TABLES 19 and 20.

TABLE 19. Contents of Carbohydrates (A)—San-Bi (on dry basis, %)

Position		Total Sugar	Sol. Sugar	Red. Sugar	Non-red. Sugar	Starch
Leaf	I	—	—	—	—	—
	II	8.64	3.58	2.70	0.88	5.06
	III	9.14	3.79	3.07	0.72	5.35
	IIII	9.30	5.29	3.95	1.34	4.01
	V	11.36	7.14	5.70	1.44	4.22
Stem	I	23.85	18.99	5.88	13.12	4.86
	II	27.30	23.14	5.65	17.49	4.16
	III	28.09	20.10	4.91	15.19	7.99
	IIII	25.20	19.38	6.79	12.59	5.82
	V	—	—	—	—	—

TABLE 20. Contents of Carbohydrates (B)—Aka-Hen (on dry basis, %)

Position		Total Sugar	Sol. Sugar	Red. Sugar	Non-red. Sugar	Starch
Leaf	I	—	—	—	—	—
	II	12.34	8.19	6.23	1.91	4.15
	III	12.50	8.80	6.53	2.27	3.70
	IIII	16.05	11.16	8.81	2.35	4.89
	V	17.91	14.71	10.22	4.49	3.20
Stem	I	31.17	24.23	7.91	16.32	6.94
	II	33.42	27.42	7.30	20.12	6.00
	III	35.61	25.96	9.48	16.48	9.65
	IIII	32.70	24.02	12.19	11.83	8.68
	V	—	—	—	—	—

4. **Total nitrogen:** We used the modified semimicro Kjeldahl method[61] of *total nitrogen* as the following:-

Manipulation. Take 0.5g. of the sample, 3g. of the digestion accelerator (*copper sulfate* : *potassium sulfate* = 1 : 9), and 5cc. of concentrated *sulfuric acid* into the digestion flask (70cc.). The mixture was heated slowly to decompose to dark-brown → orange-brown → clear pale yellowish green color after half an hour. The heating was continued 30 minutes more to digest completely. (The decomposed vapors of *sulfuric acid* were escaped to the running water through the fume pipe of glass attached to an aspirator.)

The digested liquid was cooled and diluted with a little water and transformed to the 100cc.-mess-flask to the mark. Take A cc. (generally 10~20cc.) from the above liquid into the distilling flask and added 30%-*NaOH* solution (about 15~25cc.) to strong alkaline mixture. Then distilled with steam, the *ammonia* evolved in steam was absorbed in *a* cc. of N/50-H_2SO_4. After the distillation was over (about 8 minutes) the excess *sulfuric acid* was titrated to neutral with *b* cc. of *N*/100-*NaOH*

solution when boiled for 1~3 minute and in the hot state in presence of *methyl red* as an indicator.

If *a* cc. of *N*/50-H_2SO_4 corresponds to *d* cc. of N/100-*NaOH*, and x (mg.) be the quantity of *nitrogen* per 1cc. of N/100-*NaOH*, we have the next relation:-

Nitrogen in A cc. $= x(d-b)$ mg.

where x may be calculated from the following two equations (i,ii) as 0.140 mg.

$$H_2SO_4 + 2NaOH = Na_2SO_4 + 2H_2O \cdots\cdots\cdots\cdots (i)$$

$$H_2SO_4 + 2NH_3 = (NH_4)_2SO_4 \cdots\cdots\cdots\cdots\cdots\cdots (ii)$$

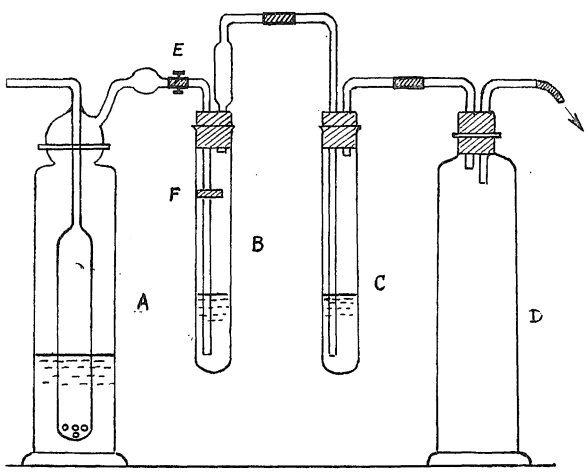
(N.B. We prepared the N/50-H_2SO_4 solution by titration in hot state with a standard solution of N/50-Na_2CO_3 using *methyl red* as an indicator.)

5. **Proteins:** It is obtained from the *total nitrogen* multiplied with 6.25 (the average content of *nitrogen* in proteins is 16%).

6. **Soluble nitrogen** : Weigh 5g. of the sample to the 250cc.-mess-flask and added 80cc. of distilled water, 10cc. of 1%-*sodium tungstate*, and 10cc. of 2/3N-H_2SO_4. After stand over night we made it to 250cc. and filtered. We used the definite volume(25 or 50cc.)of the filtrate for the *soluble nitrogen* by Kjeldahl's method.

7. **Proteinous nitrogen** : We calculated the difference from *total nitrogen* to *soluble nitrogen*.

8. **Ammoniacal nitrogen** : We used the definite volume of the filtrate for analyzing *soluble nitrogen* through Folin' s aeration method[58] (FIG.10).



A : Dil.-H_2SO^4; B : Sample solution ; C : Normal acid
D : Safety bottle; E : Pinch-cock; F : Gum plate
FIG.10. Apparatus for Ammonia aeration method

Taking 10cc.of the filtrate into the distilling tube B ($2.5\times20cm^2$) with 1~2 drops of *phenolphthalein* solution and dipped in the water bath at 40~50°C. The absorbing tube C, in which the liquid layer was made up to 5 cm. by adding 3cc. of N/50-H_2SO_4 and 10cc. of water, was connected to B. Into the liquid in B, which we sucking gently with aspirator, we added 0.5cc. of a reagent (10

g.of anhydrous *sodium carbonate*, 15 g. of *potassium oxalate*, and distilled water to the 100cc. solution) to weak alkaline state. Then sucked it faintly for 3 minutes and again strongly. At last, it was distilled in vacuum for 3 minutes after shutting with the pinchcock E, and the liberated *ammonia* was absorbed with the standard *sulfuric acid* in the tube C. The time of aeration is enough for 30 minutes. The contents of the absorption tube C was transformed to the 200cc.-Erlenmeyer flask and titrated as usual for the *nitrogen* with N/100-*NaOH* solution using *methyl red* as an indicator. The results are shown in the TABLES 21 and 22.

TABLE 21. Contents of Nitrogen Compounds (A) — San-Bi (on dry basis, %)

Position		Total-N	Protein-N	Sol.-N	NH_3-N
Leaf	I	—	—	—	—
	II	2.910	2.513	0.397	0.037
	III	3.528	3.014	0.514	0.065
	IIII	3.981	3.192	0.789	0.101
	V	4.353	3.281	1.072	0.109
Stem	I	0.757	0.584	0.173	—
	II	0.866	0.643	0.223	—
	III	1.047	0.808	0.239	—
	IIII	1.458	1.217	0.241	—
	V	2.089	—	—	—

TABLE 22. Contents of Nitrogen Compounds(B) — Aka-Hen (on dry basis, %)

Position		Total-N	Protein-N	Sol.-N	NH_3-N
Leaf	I	—	—	—	—
	II	2.175	1.994	0.181	0.019
	III	2.445	2.204	0.241	0.058
	IIII	2.976	2.544	0.432	0.062
	V	3.433	2.828	0.605	0.078
Stem	I	0.567	0.431	0.136	—
	II	0.641	0.450	0.196	—
	III	0.763	0.557	0.206	—
	IIII	0.998	0.789	0.209	—
	V	1.511	—	—	—

9. **Fat**: 2~3 g.of the sample was extracted with the Soxhlet extractor using *ether* as a solvent (it was required 16 hrs.: leaves, and 8 hrs.: stems) (TABLE 23,24).

10. **Cellulose** : For the analysis of *cellulose*[34,35] by the Cross-Bevan's method we took 1~2 g. of *ether* insoluble residue. We adopted Dore's apparatus. The sample was taken to the glass filter

"1G3" (which pre-weighed) and washed with distilled water, then chlorinated (*chlorine* was evolved from $KMnO_4$ and *HCl*) through the covering funnel. The flow of the *chlorine* was adjusted for constant bubble numbers of 150~180 per minute. After the chlorination for 20 minutes, it was washed with hot water and removed again the *chlorine* with *2%-sodium sulfite* solution. Transforming, then, the contents in the filter to the 300cc. Erlenmeyer flask with 100~120cc. of *3%-sodium sulfite* solution, and boiled for 15 minutes.

By repeating the treatments with washing, chlorination, and *sodium sulfite* digestion, the sample became nearly white. This white substance was bleached with 20cc. of *0.1%-$KMnO_4$* solution and washed with sulfite solution, and again with the large quantity of hot water (over 2 liters). It was washed to the last with *95% ethanol* and dried at 105°C. to the constant weight (cf. A.O.A.C. method[54]). These results are shown in the following TABLES 23 and 24.

TABLE 23. Contents of Organic Substances (A)—San-Bi (on dry basis, %)

Position		Carbohydrate	Protein	Fat	Cellulose
Leaf	I	—	—	7.09	—
	II	8.64	18.19	5.86	18.2
	III	9.14	22.06	6.24	17.1
	IIII	9.34	24.88	5.76	14.3
	V	11.36	27.19	5.69	12.3
Stem	I	23.85	4.75	0.78	34.7
	II	27.30	5.38	0.67	31.0
	III	28.09	6.56	1.26	27.1
	IIII	25.20	9.13	1.66	24.2
	V	—	13.06	1.67	19.6

TABLE 24. Contents of Organic Substances (B)—Aka-Hen (on dry basis, %)

Position		Carbohydrate	Protein	Fat	Cellulose
Leaf	I	—	—	—	—
	II	12.34	13.63	5.79	16.0
	III	12.50	15.25	5.56	14.2
	IIII	16.05	18.63	4.94	11.3
	V	17.91	21·44	5.30	10.0
Stem	I	31.17	3.56	0.48	30.7
	II	33.42	4.00	0.70	28.9
	III	35.61	4.75	0.64	24.2
	IIII	32.70	6.25	0.99	20.2
	V	—	9.44	1.75	16.8

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