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Seasonal Change of Sapogenin Content in Third-Year Plants of *Dioscorea tokoro* Makimo[1]

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To examine the seasonal changes of the sapogenins contained in *Dioscorea tokoro* Makino, third-year plants were cultivated on an experimental farm. Rhizome weight started to increase at the end of August, when rapid shoot growth was arrested. Rhizome enlargement was not observed in female plants. The concentrations and quantities of the sapogenins differed with the morphological part of the plant and the month of harvest. Yonogenin quantities in leaves and flowers continued to increase until the end of summer, after which its concentration was maintained in the leaves but decreased rapidly in the flowers. Most of the yonogenin in the aerial parts was degraded or transformed into other substances during September and November. Tokorogenin was present at much lower concentrations in all parts and at all times. Diosgenin was generally found in the rhizome as sugar-bound saponins, and at markedly high levels during August and October. However, four of the 84 plants used contained large quantities of diosgenin in the leaves and flowers.

Keywords——*Dioscorea tokoro*; steroidal sapogenin; diosgenin; yonogenin; tokorogenin; growth; seasonal change; distribution

Diosgenin (25D-spirost-5-en-3β-ol), a starting material for the synthesis of steroid hormones, is isolated from rhizomes or tubers of several wild Dioscorea plants. However, the steady upward demand for raw materials has made supplies of these wild yams scarce and resulted in experimental cultivation. The sapogenin-containing organs of the plants, the underground parts, are perennial and continue to grow year by year, although the stems and leaves wither in autumn in the temperate region or in the dry season in the tropics. The variation in the condition of the aerial parts appears to influence the sapogenin content in the underground parts, as steroidal sapogenins are generally considered to be synthesized in the aerial parts and rapidly transported to the underground parts.[3] Studying the seasonal changes of sapogenin Cruzado, *et al.*[4] found that the sapogenin content in tubers of *Dioscorea composita* Hemsl. and *D. floribunda* Mart. et Gal. generally increased with plant age, without marked seasonal fluctuation, though the increase was more rapid in summer and autumn and slower or even exhibited a slight reverse in spring. Karnick[5] also observed similar trends in *D. deltoidea* Wall. and *D. prazeri* Prain et Burkill. The results of Blunden, *et al.*[6] differed slightly. They also observed the increase of sapogenin concentration with tuber

1) Studies on the Steroidal Components of Domestic Plants. LXVII. Part LXVI: T. Okanishi, A. Akahori, F. Yasuda, Y. Takeuchi and T. Iwao, *Chem. Pharm. Bull.* (Tokyo), **23**, 575 (1975). Part of the present work was reported at the annual meeting of the Japanese Society of Pharmacognosy at Nagasaki in 1972.

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3) R.D. Bennet, E. Heftmann and W.H. Preston, Jr., *Arch. Biochem. Biophys.*, **103**, 74 (1963).

4) H.J. Cruzado, H. Delpin and B.A. Roark, *Turrialba*, **15**, 25 (1965).

5) C.R. Karnick, *Planta Med.*, **16**, 269 (1968).

6) G. Blunden, R. Hardman and F.J. Hind, *Planta Med.*, **19**, 19 (1970).

age both in *D. hondurensis* R. KNUTH and *D. sylvatica* ECKLON but found different patterns of seasonal variation. The concentration decreased in March when new shoots emerged in *D. hondurensis*, but continued to increase in *D. sylvatica*. These plants contained the sapogenins almost exclusively in their underground parts with none or only a trace in their aerial parts. This stimulated us to investigate the seasonal changes in *D. tokoro* MAKINO which contains sapogenins in both parts.

Previously,[7] we reported the changes in the sapogenins of plants in the first year of growth. Isodiotigenin (25D,5β-spirostane-2β,3α,4β-triol), which had not been found in mature plants, appeared soon after germination and gradually increased in quantity. The increase of yonogenin (25D,5β-spirostane-2β,3α-diol), always found in large amounts in the aerial parts of mature plants occurred a little later. These changes in sapogenin content were considered to be affected by seedling maturation and the change of environmental conditions. This time we studied the seasonal variations of the sapogenins, excluding the dramatic change in the early stage of plant development.

Materials and Methods

Materials——Seeds of *D. tokoro* were collected in October at the experimental farm of Aburahi Laboratories, stored in a desiccator and germinated in petri dishes in April of the next year. Seedlings were planted in a nursery bed ten days after emergence. They were transplanted in October of the next year in the field in rows 50 cm apart. Plants were spaced at 30 cm intervals in rows in black forest soil. Bamboo poles were used as vine supports. Fertilizer was not supplied.

TABLE I. Numbers of Plants Used

Date harvested	Sex		
	Male	Female	Total
15 June 1967			15[a]
15 July	6	4	15[b]
15 Aug.	11	4	15
13 Sept.	5	10	15
16 Oct.	11[c]	3	14
15 Nov.	12	3	15
15 Feb. 1968	13	2	15
15 April	9	6	15

a) No flowers were observed.
b) Five plants did not bear flowers.
c) One plant, the aerial parts of which died by mechanical injury, was excluded.

The plants were harvested in their third season of growth, fifteen each month as summarized in Table I. The samples were immediately separated into four parts, rhizome plus root, leaf, stem, and flower, then weighed. Axes of inflorescences, pedicels of flowers and capsules were included in the flower part. Inflorescences and leaves were separated from stems at the bases of their axes and petioles, respectively. Each part was dried at 70° to constant weight; rhizomes after being cut into pieces, were weighed and stored in a desiccator.

Like other species of this genus, although this plant is dioecious, there is no morphological difference other than the flowers between the male and female plants. Therefore, it was impossible to know the sex of the individuals at the beginning of the third season, as most had not borne flowers the year before. This caused a marked unbalance between the numbers of male and female samples.

Extraction of Sapogenins——About two grams of each dried sample was weighed exactly, extracted with methylene chloride and then methanol in a Soxhlet apparatus. The methylene chloride and methanol extracts were treated as free and sugar-bound sapogenin fractions, respectively. The methanol extracts were hydrolyzed in 5% HCl for 5 hr under reflux. The hydrolysis products were extracted with ethyl

7) A. Akahori, F. Yasuda, I. Okuno, M. Togami, T. Okanishi and T. Iwao, *Phytochem.*, **8**, 45 (1969).

TABLE II. Dry Weights of the Samples (g)

Part	Sex	Month harvested															
		June		July		Aug.		Sept.		Oct.		Nov.		Feb.		April	
		No.	Weight	No.	Weight	No.	Weight	No.	Weight	No.	Weight	No.	Weight	No.	Weight	No.	Weight
Leaf	Male			6	4.5±1.3	11	13.8±5.6	5	15.7± 7.5	11	23.1±10.8	12	4.8± 3.6				
	Female			4	8.3±2.8	4	11.5±1.8	10	13.2± 5.0	3	9.3± 5.0	3	5.9± 5.9				
	Unknown	15	1.0±0.7	5	4.9±1.8	0		0		0		0					
	Total	15	1.0±0.7	15	5.6±2.4	15	13.2±5.0	15	14.0± 5.8	14	20.2±11.4	15	5.0± 3.9	0		0	
Stem	Male			6	4.4±1.1	11	8.9±3.4	5	10.3± 4.3	11	14.7± 7.6	12	7.9± 4.3				
	Female			4	8.2±2.8	4	6.9±2.0	10	9.4± 3.4	3	6.0± 3.4	3	6.0± 1.7				
	Unknown	15	1.4±0.8	5	4.7±2.0	0		0		0		0					
	Total	15	1.4±0.8	15	5.5±2.5	15	8.4±3.2	15	9.7± 3.6	14	12.8± 7.6	15	7.5± 3.9	0		0	
Flower	Male			6	1.2±1.1	11	7.7±2.9	5	14.1± 6.9	11	9.8± 6.8	12	4.9± 2.6				
	Female			4	0.3±0.3	4	7.1±2.5	10	38.0±12.0	3	39.4± 5.4	3	36.2±18.2				
	Unknown					0		0		0		0					
	Total	0		10	0.8±0.9	15	7.5±2.7	15	30.0±15.6	14	16.1±14.1	15	11.2±14.4	0		0	
Rhizome	Male			6	4.6±1.8	11	7.7±2.6	5	26.5±16.6	11	51.6±17.4	12	48.4±19.3	13	47.0±13.9	9	50.6±19.2
	Female			4	4.9±1.4	4	4.7±1.5	10	7.9± 4.9	3	5.2± 3.4	3	18.4±18.0	2	8.7± 2.9	6	9.7± 4.9
	Unknown	15	3.2±0.9	5	3.8±1.4	0		0		0		0		0		0	
	Total	15	3.2±0.9	10	4.4±1.5	15	6.9±2.7	15	14.1±13.3	14	41.7±25.0	15	42.4±22.2	15	41.9±18.7	15	34.2±25.5

TABLE III. Concentrations of Steroidal Sapogenins (% of Dry Weight)

Sapogenin	Type	Part	Month harvested No. June[a] 15	July 6	Aug. 11	Sept. 5	Oct. 11	Nov. 12	Feb. 13	April 9
1. Male plants										
Yonogenin	Free	Leaf	2.61±0.61[b]	1.64 ±0.56	2.72 ±0.62	2.27 ±0.62	0.58 ±0.29[c]	0.07 ±0.06		
		Stem	0.09±0.04[b]	0.16 ±0.03	0.09 ±0.03	0.06 ±0.02	0.02 ±0.02	0.003±0.003		
		Flower	—	3.90 ±0.62	0.92 ±0.44	0.90 ±0.29	0.39 ±0.26	0.45 ±0.14		
		Rhizome	0	0.001±0.001	0.005±0.003	0.007±0.002	0.008±0.003	0.005±0.003	0	0.003±0.001
	Bound	Leaf	0.28±0.18	0.33 ±0.17	1.06 ±0.38	0.39 ±0.16	0.19 ±0.29	0.05 ±0.03		
		Stem	0.19±0.08[d]	0.16 ±0.09	0.06 ±0.03	0.04 ±0.01	0.01 ±0.01	0.002±0.002		
		Flower	—	0.71 ±0.54	1.11 ±0.21	0.30 ±0.11	0.14 ±0.13	0.08 ±0.03		
		Rhizome	0.04±0.01[d]	0.015±0.012	0.02 ±0.01[e]	0.03 ±0.01	0.02 ±0.01[e]	0.02 ±0.01[e]	0.02 ±0.01	0.02 ±0.01
Tokorogenin	Free	Leaf	0.13±0.05[b]	0.07 ±0.04	0.18 ±0.07	0.34 ±0.04	0.28 ±0.07[c]	0.05 ±0.03		
		Stem	0.01±0.01[b]	0.02 ±0.01	0.018±0.004	0.016±0.004	0.008±0.002	0.001±0.001		
		Flower	—	0.08 ±0.02	0.07 ±0.02	0.25 ±0.05	0.15 ±0.04	0.16 ±0.04		
		Rhizome	0	0.002±0.003	0.003±0.002	0.002±0.001	0.003±0.001	0.001±0.001	0	0.002±0.001
	Bound	Leaf	0.12±0.08	0.04 ±0.02	0.16 ±0.05	0.12 ±0.05	0.19 ±0.02	0.11 ±0.04		
		Stem	0.18±0.05[d]	0.19 ±0.05	0.18 ±0.04	0.17 ±0.03	0.06 ±0.02	0.13 ±0.01		
		Flower	—	0.07 ±0.003	0.22 ±0.08	0.26 ±0.07	0.19 ±0.05	0.11 ±0.02		
		Rhizome	0.17±0.02[d]	0.12 ±0.004	0.19 ±0.02[e]	0.17 ±0.03	0.13 ±0.02[e]	0.08 ±0.02	0.13 ±0.03	0.14 ±0.05
Diosgenin	Bound	Rhizome	0.63±0.18[d]	0.56 ±0.41	0.61 ±0.15[e]	0.55 ±0.12	0.56 ±0.12[e]	0.35 ±0.10	0.40 ±0.18	0.73 ±0.29
2. Female plants										
Yonogenin	Free	Leaf		1.88 ±0.70	3.15 ±0.25	1.93 ±0.31	0.38 ±0.16[f]	0.04 ±0.01		
		Stem		0.14 ±0.04	0.07 ±0.03	0.04 ±0.01	0.008±0.003	0.001>		
		Flower		3.80 ±0.69	2.11 ±1.10	0.67 ±0.18	0.11 ±0.02	0.01 ±0.01		
		Rhizome		0.003±0.003	0.005±0.003	0.002±0.001	0	0.002±0.001	0	0.002±0.002
	Bound	Leaf		0.23 ±0.07	0.99 ±0.30	0.36 ±0.19	0.16 ±0.08	0.03 ±0.01		
		Stem		0.10 ±0.03	0.054±0.004	0.03 ±0.01	0.012±0.001	0.001±0.002		
		Flower		0.80 ±0.37	0.46 ±0.10	0.07 ±0.02	0.03 ±0.01	0.008±0.001		
		Rhizome		0.02 ±0.01	0.024±0.004	0.03 ±0.01	0.02 ±0.01	0.019±0.005	0.037±0.003	0.02 ±0.01
Tokorogenin	Free	Leaf		0.08 ±0.03	0.21 ±0.04	0.40 ±0.14	0.21 ±0.02[f]	0.04 ±0.01		
		Stem		0.02 ±0.01	0.015±0.001	0.014±0.003	0.008±0.002	0.001>		
		Flower		0.09 ±0.05	0.08 ±0.03	0.13 ±0.05	0.06 ±0.02	0.01 ±0.01		
		Rhizome		0.002±0.002	0.003±0.002	0.001±0.001	0.003±0.001	0.001±0.001	0	0.002±0.002
	Bound	Leaf		0.04 ±0.02	0.18 ±0.05	0.16 ±0.06	0.28 ±0.05	0.07 ±0.01		
		Stem		0.15 ±0.01	0.18 ±0.03	0.18 ±0.01	0.13 ±0.02	0.02 ±0.01		
		Flower		0.09 ±0.02	0.12 ±0.02	0.16 ±0.01	0.15 ±0.07	0.06 ±0.01		
		Rhizome		0.20 ±0.01	0.21 ±0.03	0.17 ±0.03	0.25 ±0.07	0.07 ±0.03	0.26 ±0.08	0.20 ±0.06
Diosgenin	Bound	Rhizome		0.67 ±0.19	0.51 ±0.05	0.70 ±0.15	0.55 ±0.11	0.16 ±0.06	0.71 ±0.22	0.51 ±0.16

Mean ± S.D. a) Sex of plants unknown. n=b, 14; c, 9; d, 13; e, 10; f, 2.

acetate. The methylene chloride extracts and hydrolysis products were saponified in 80% methanol containing 5% KOH on a water bath for 1 hr and extracted into ethyl acetate.

Determination of Sapogenin Quantities——Sapogenins were dissolved in methanol and spotted on silica gel G plates (20×20×0.03 cm). The quantities of diosgenin and other sapogenins were estimated using the methods of Akahori, *et al.*[8] and Okanishi and Togami,[9] respectively.

Results

Dry weights and water contents of the plants are given in Table II and Fig. 1, respectively. Leaf and stem weights increased rapidly during July and August and then slowly until October. Rhizome weight increased only slightly in summer. Rapid enlargement started at the end of August and continued until October in male plants. This did not occur in female plants. The water contents of leaf and stem were constant until October and those of flower until September, after which they decreased. Rhizome water content did not change until September and, after a decrease once in October, recovered slowly.

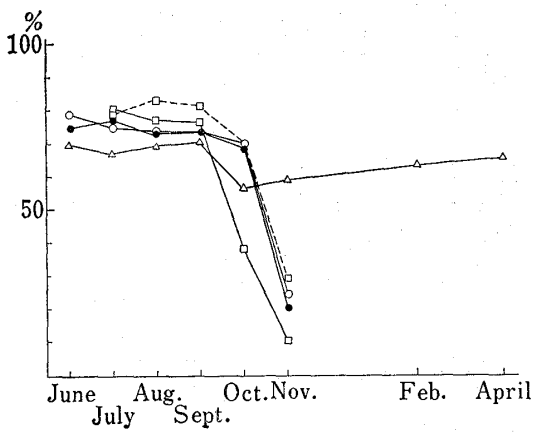

Fig. 1. Water Contents of the Male Samples

●—●, leaf. □—□, flower(male). △—△, rhizome.
○—○, stem. □---□, flower(female).

The concentrations of free and sugar-bound sapogenins in male and female plant parts are summarized in Table III. The seasonal change of sapogenin quantities is graphed in Fig. 2. No significant differences were observed between the concentrations in the corresponding parts of male and female plants, except for the flower.

The proportions and concentrations of the sapogenins varied amomg different parts of the same plants. Yonogenin was the major sapogenin in leaf and flower, but occurred in lesser quantities in the stem, with only in a minute amount in the rhizome. Diosgenin was not found in the aerial parts other than in the four plants given in Table IV. It was almost exclusively contained in the rhizome, where other sapogenins were found in much lower concentrations. Tokorogenin was not as extremely localized. Isodiotigenin was found in five plants harvested in June, five in July, three in August, two in September and one in October, mostly at a concentration of less than 0.05% of the dry weight. It was not detected in the flower and the rhizome.

The proportions of free and sugar-bound sapogenins also varied among the different parts. Much more yonogenin existed as the free than as the bound form in leaf and flower, while no significant difference was found between the concentrations of the two types in the stem. In the case of tokorogenin, the proportions of the bound type were greater than those of yonogenin for all parts and times. Diosgenin was practically found only as the bound type.

The sapogenins changed in quantity and concentration during the experiment. Yonogenin in leaf continued to increase in quantity until August, while its concentration peaked in August after decreasing once in July. Its content in the stem was highest in July and then decreased. In the flower, it continued to increase until September, after the concentration had peaked in July then rapidly decreased. The seasonal change of tokorogenin was not as prominent

8) A. Akahori, K. Murata, F. Yasuda, S. Nagase, M. Togami and T. Okanishi, *Shionogi Kenkyusho Nempo*, **16**, 74 (1966).
9) T. Okanishi and M. Togami, *Chem. Pharm. Bull.* (Tokyo), **17**, 315 (1969).

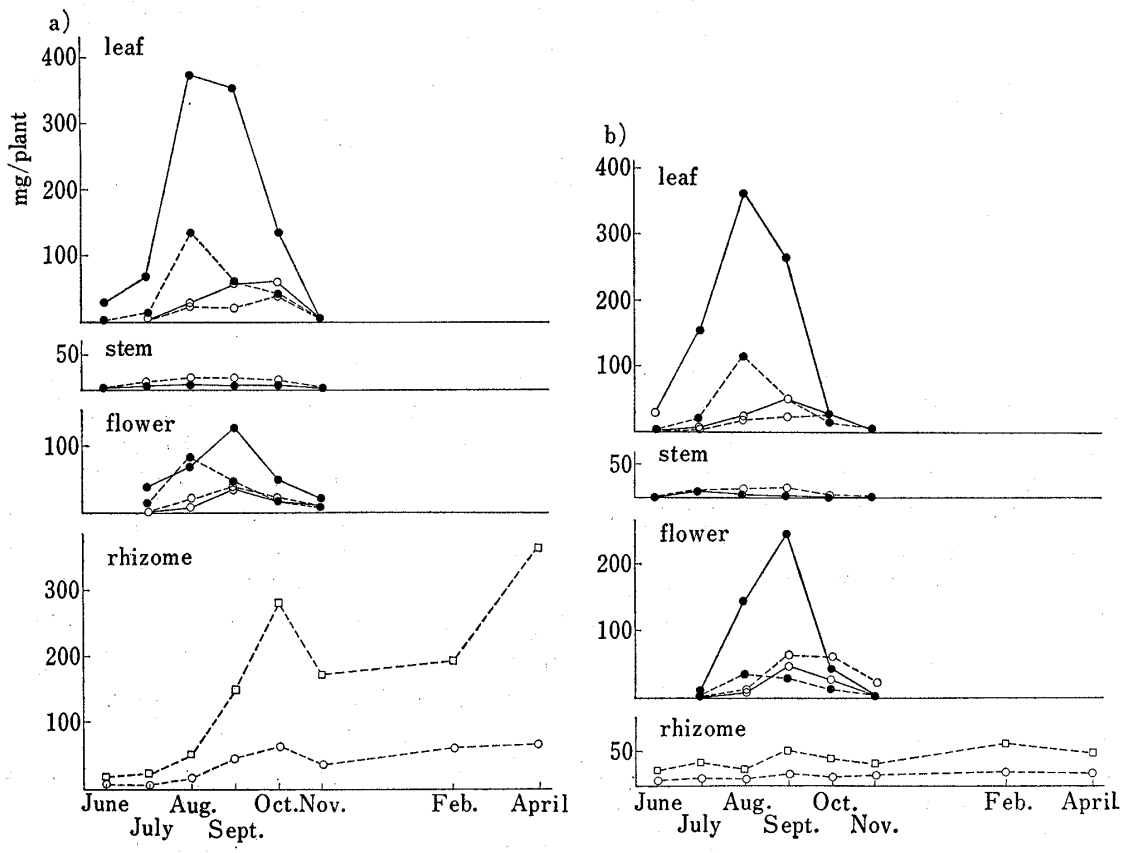

Fig. 2. Quantities of Sapogenins in Male and Female Plants

a) Male plants, b) Female plants.
The sex of plants harvested in June was unknown.
●—●, yonogenin (free). ○—○, tokorogenin (free). □---□, diosgenin (bound).
●---●, yonogenin (bound). ○---○, tokorogenin (bound).

TABLE IV. Concentrations and Quantities of Sapogenins in Plants with Diosgenin in Their Aerial Parts

Sample number	Sex	Month harvested	Part	Dry weight g	Concentration, %			Quantity, mg		
					Dios	Yono	Tokoro	Dios	Yono	Tokoro
203	M	July	Leaf	4.8	0.06	2.03	0.10	2.7	97.2	4.8
			Stem	4.2	0	0.32	0.16	0	12.2	5.9
			Flower	2.0	0.05	4.99	0.16	0.9	100.7	3.2
			Rhizome	6.8	0.19	0.001	0.06	13.3	0.1	4.2
509	M	Oct.	Leaf	25.1	0.90	0.31	0.34	225.3	78.3	85.2
			Stem	13.4	0	0.03	0.09	0	3.4	11.1
			Flower	6.5	0.13	0.21	0.26	8.3	13.4	17.1
			Rhizome[a)]	62.6	—	—	—	—	—	—
601	F	Nov.	Leaf	4.4	0.90	0.06	0.10	39.6	2.6	4.3
			Stem	5.7	0	0	0.01	0	0	0.8
			Flower	50.8	0	0.01	0.06	0	4.6	28.3
			Rhizome	10.5	0.22	0.02	0.04	23.4	2.4	4.3
605	M	Nov.	Leaf	3.2	0.99	0.06	0.14	31.6	2.1	4.6
			Stem	5.6	0	0.004	0.01	0	0.2	0.7
			Flower	2.1	0	0.41	0.22	0	8.6	4.6
			Rhizome	40.6	0.18	0.03	0.08	71.6	14.0	33.7

Abbreviation: M, male; F, female.
a) Determination of the sapogenin quantities failed.

as that of yonogenin. The maximum quantities and concentrations of the former were recorded a little later in the leaf and stem than those of the latter. The tokorogenin concentration did not vary in the flower. The quantity of diosgenin in the rhizome of male plants did not change markedly during June and July. It started to increase in August, slowly at first, then rapidly until October and, after decreasing once in November, increased again. No marked variation was recorded in its concentration except for a decrease in November.

Discussion

Our previous study on the sapogenin composition of *D. tokoro*[7] seedlings presented evidence that synthesis of the sapogenin is affected markedly by plant age. This influence of plant maturation seems to be arrested in the third season, which lead us to study the third-year plants. Isodiotigenin, which is considered to be synthesized when new individuals are formed due to germination[7] and budding from rhizomes cut into small pieces,[10] was practically not detected in leaves of third-year plants. However, the unbalance between the numbers of male and female plants resulted in some cases, in an insufficient number of samples to estimate the mean values of the corresponding populations. The wide variance inherent to wild plants as seen in Tables II and III also made analysis of some data difficult.

As summarized in Table II, the shoots continued to develop until October. However, the slight decrease in water content in the leaf and stem in October suggests that the withering process already had started in these parts at the time of their full development. Their marked decrease in weight and water content in November results partly from the loss of withered leaves and partly from the disappearance of cell components due to translocation of the assimilation products from the aerial to the underground parts and/or their decomposition into lower molecules such as carbon dioxide and water. Stable dry weights of the flower in female plants through autumn suggests that the cell components remained during fruit ripening, although water was mostly lost. Like tubers of edible yams,[11] the rhizomes began enlarging in male plants when the growth of the aerial parts slowed down, and stopped in November when the aerial parts died. This also suggests that the assimilation products synthesized in leaves and used for shoot development in summer were translocated in autumn down through the stems for rhizome growth. The rapid enlargement of the rhizome was not recorded in female plants, probably because the nutrients were used for fruit ripening. This marked retardation of development of the underground storage organ, which is estimated to occur generally in Dioscorea plants, probably affects the growth of female plants in the following year; fewer female than male plants have been found in the field.[12]

Proportions of the sapogenins differ among the different parts,[13] as in other plants.[14] Seasonal difference was also found; the quantities of each sapogenin and their proportions fluctuated markedly. Yonogenin is localized in the assimilative and reproductive organs, with over 90% in the leaf and flower, except in November. Its rather stable concentration in the leaf in summer suggests that the leaf continues to synthesize it together with other cell components until the development of the leaf arrest, and retains it as long as the function of the leaf remains. In the flower, the yonogenin concentration peaked in July then decreased rapidly. Flower buds formed and developed successively with shoot elongation from

10) A. Akahori, F. Yasuda, M. Togami, K. Kagawa and T. Okanishi, *Phytochem.*, **8**, 2213 (1969).
11) D.G. Coursey, "Yams," Longmans, 1967, p. 73; R.A. Sobulo, *Exp. Agric.*, **8**, 99 (1972).
12) D.G. Coursey, "Yams," Longmans, 1967, p. 35.
13) A. Akahori, *Shionogi Kenkyusho Nempo*, **11**, 93 (1961); *ibid.*, **13**, 68 (1963); Akahori, I. Okuno, T. Okanishi and T. Iwao, *Chem. Pharm. Bull.* (Tokyo), **16**, 1994 (1968).
14) E. Heftmann, *Lloydia*, **30**, 209 (1967); G. Blunden, Yi Yi and K. Jewers, *Lloydia*, **37**, 10 (1974); M.M. El-Olemy, J.J. Sabatka and S.J. Stohs, *Phytochemistry*, **13**, 489 (1974).

July until the beginning of September. The flower part harvested in July was almost entirely composed of buds and that collected in September, withered flowers or unripened capsules mixed with a few blooming flowers and buds. Thus, the concentration of yonogenin in buds should not be lower than that recorded in July and that in withered flowers less than that in September. The high concentration of yonogenin in buds seems to be partly due to the tissues having smaller cells per unit of flower area.[15] Its concentration did not increase markedly in flowers as they developed. Assimilates from mature leaves are said to move into immature ones with little movement between mature leaves.[16] Others have reported that metabolite transferred upward from young leaves of the bean plant is rich in steroid and that downward from mature leaves rich in sucrose.[17] Therefore, yonogenin seems to be translocated from the leaves at an early stage of flower formation rather than being synthesized in the buds. The ratios of free to bound yonogenin, which differed with the morphological part and did not markedly change throughout the experiment, probably depend on the physiological functions of the two types.

The concentrations of tokorogenin did not vary as markedly among the parts of the same plant or samples of the corresponding parts harvested in different months. They were almost constant in the rhizome throughout the year. The development of each part does not seem to affect tokorogenin synthesis.

Diosgenin, which has been considered as being practically nonexisting in the aerial parts of the plant,[13,18] was found in the leaf and flower of four samples (Table IV). Its concentrations in leaves of three plants harvested in October and November were 0.90, 0.90 and 0.99% to dry weight and much higher than those of other sapogenins in the aerial parts and those of all sapogenins in the rhizome. The concentrations of other sapogenins and dry weights of each part of these four plants did not differ significantly from those of the other samples harvested at the same time. Its concentration change in the rhizome agrees with those in literature.[4-6]

Most of the yonogenin and about two-thirds of the tokorogenin were lost from the plants during September and November, as shown in Figure 2. The decrease in total tokorogenin seems to be mostly due to the fall of the leaves and flowers, because its concentration in these parts did not change markedly at this time. In contrast to tokorogenin, the rapid decrease of yonogenin concentration in leaf at the beginning of autumn indicates that it was either translocated to other morphological parts or decomposed and transformed into other substances. The marked increase of diosgenin in rhizome at the same time might suggest that this sapogenin is derived from yonogenin, directly or indirectly. Although diosgenin usually exists in the underground parts of sapogenin-containing Dioscorea plants in much higher concentrations than other sapogenins,[19] it was found only in trace amounts in the rhizomes of a clone of *D. tenuipes* Franch. et Savat. derived from a plant collected at Hitoyoshi.[20] Furthermore, tokorogenin which was isolated together with neotokorogenin from the rhizomes, was not found in the aerial parts. This led us to postulate that the sapogenins are synthesized in both parts of the plant and not translocated from one part to another, or alternatively, conversion of the side chains occurs either in the aerial or underground parts. Although the results of the present work do not entirely contradict those of previous studies,[20] diosgenin is probably at least partly synthesized in the leaves; high

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concentrations recorded in leaves No. 509, 601 and 605 may have resulted from accumulation of the sapogenin where it was synthesized due to blocking of its transport to the rhizomes. The insufficient number of samples does not allow any conclusion, but the low concentration in leaf No. 203 harvested in July supports the above hypothesis and suggests that active synthesis of diosgenin in leaves starts at the end of summer. The trace amount of diosgenin sometimes detected in the aerial parts[13,18] is considered to be due to migration of individuals containing this sapogenin like the above four plants into the extraction material. The decrease of yonogenin in leaf and increase of diosgenin in rhizome at the end of summer and the middle of autumn agree with our previous report on the sapogenin concentration being maintained under short-day conditions[21] and suggest that this was at least partly caused by shortening the day length.

In spite of the hypotheses proposed by many investigators, the physiological role of the steroidal sapogenins or saponins has not been elucidated. However, their synthesis in growth and degradation in the withering stage suggest that they participate in the metabolic function of the cells.

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