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# Studies on the Steroidal Components of Domestic Plants. LIX.[1] Effect of Light on the Steroidal Sapogenins of *Dioscorea tokoro* MAKINO

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The second-year plants of *Dioscorea tokoro* MAKINO were maintained under short-day (8 hr light and 16 hr dark) or long-day conditions (16 hr light and 8 hr dark) for a month. The amount of yonogenin and tokorogenin of the aerial parts was markedly increased together with an increase in the weight of these parts by elongating the photoperiod, while the concentration of isodiotigenin was decreased by this treatment. Light conditions did not effect on the underground parts of the plant.

Interest in the relationships between light and plant growth is reflected by the large number of publications on the subject. On the effect of light on the growth and development of plants, three primary factors have been considered, the intensity, the wavelength of the light, and the duration of the light exposure. Since Garner and Allard[3,4] emphasized the improtance of day length in the formation of flower buds, investigation in this field has developed intensively. Changes in the chemical composition of plants caused by control of the photoperiod have also been reported. Recently, an interesting report was presented by Grahle and Höltzel[5] which stated that the essential oil of the leaves of *Mentha piperita* L. maintained under long day-light conditions was almost completely composed of menthone and menthol, while under short day-light conditions, it was consisted mainly of menthofuran. Burbott and Loomis[6] observed a similar phenomenon with the monoterpenes of the peppermint plant, but they did not entirely attribute this to the photoperiodic effect.

Since tubers of some Dioscorea species have been used as one of the major commericial sources of sapogenins for the synthesis of steroidal hormones, the cultivation of these plants and their sapogenin content have attracted world-wide attention. The sapogenin content in Dioscorea increases gradually with the age of the plant, more rapidly in summer than in winter.[7,8] The composition and concentration of the sapogenins in *D. tokoro* MAKINO changes markedly both with the age of the plant[9] and with the plant growth stage.[10,11] However, the concentration of diosgenin in the aerial parts of this plant[1,10,11] and other Japanese Dios-

1) Part LVIII: A. Akahori, F. Yasuda, M. Togami, K. Kagawa and T. Okanishi, *Phytochemistry*, **8**, 2213 (1969).
2) Location: *a*) *Fukushima-ku, Osaka*; *b*) *Koga-cho, Shiga.*
3) W.W. Garner and H.A. Allard, *J. Agr. Res.*, **18**, 553 (1920); W.W. Garner, C.W. Bacon and H.A. Allard, *ibid.*, **27**, 119 (1924); W.W. Garner and H.A. Allard, *ibid.*, **31**, 555(1925).
4) W.W. Garner and H.A. Allard, *J. Agr. Res.*, **23**, 871 (1923).
5) A. Grahle and C. Höltzel, *Naturwissenschaften*, **50**, 552 (1963).
6) A.J. Burbott and W.D. Loomis, *Plant Physiol.*, **42**, 20 (1967).
7) H.J. Cruzado, H. Delpin and B.A. Roark, *Turrialba*, **15**, 25 (1965).
8) C.R. Karnick, *Planta Med.*, **16**, 269 (1968).
9) A. Akahori, F. Yasuda, I. Okuno, M. Togami, T. Okanishi and T. Iwao, *Phytochemistry*, **8**, 45 (1969).
10) A. Akahori, *Ann. Rep. Shionogi Res. Lab.*, **13**, 68 (1963); A. Akahori, I. Okuno, T. Okanishi and T. Iwao, *Chem. Pharm. Bull.* (Tokyo), **16**, 1994 (1968).
11) A. Akahori, *Ann. Rep. Shionogi Res. Lab.*, **11**, 93 (1961).

corea species[12] is very low during the various ages and growth stages, contrary to the report by Blunden, *et al.*[13] on Himalayan and Mexican Dioscorea species cultivated in England. This discrepancy is assumed to result partly from differences in the growing conditions, to which the difference in the day length, between Osaka and Portsmouth may be contributory. This led us to examine the effect of the day length on the concentration of sapogenins.

### Experimental

**Material**——Second-year rhizomes of *D. tokoro* were seeded on July 6, 1967 at the Aburahi Laboratories and transplanted into pots on May 17, 1968. Bamboo poles were used as vine supports.

**Day-Length Control**——Experimental rooms were set up in a greenhouse. They were constructed of a wooden flamework 1.0×1.0 m wide and 1.3 m high and covered with black cloth curtains. Supplementary illumination was from two 10 watt day-light flourescent National FL 10 lamps installed in the top of the room (500 lux at the upper surface of the pot). Plants were transferred into the rooms on June 1. They received natural day light from 9 am to 5 pm (8 hr) and were then covered with shading cloths (from 5 pm to 9 am). The long day group was supplemented with artificial illumination from 5 pm to 1 am (8 hr) and the short day group was kept without light for 16 hr. These light conditions were maintained for one month. The temperature in the rooms was kept the same for both groups (30° by day and 20° by night). The plants were harvested on July 1, divided into leaf, stem and rhizome, weighed, then dried at 70° and stored in a dessicator.

**Analysis of Sapogenins**——Free sapogenins were extracted from the dried material in a Soxhlet apparatus with methylene dichloride. Saponins were then extracted with methanol. The residue after evaporation of the solvent from the methylene dichloride extracts was dissolved in 50 ml of methanol containing 2.5 g of KOH and refluxed for 1 hr. The unsaponified substance was extracted into ethyl acetate. This extract was analyzed colorimetrically by the Okanishi and Togami method.[14] The methanol solution was evaporated to dryness, then the residue was dissolved in 50 ml of water containing 7.5 ml of 35% HCl and warmed on a boiling water bath for 5 hr. The hydrolized product was extracted with ethyl acetate and analyzed for glycosidal sapogenins. The sapogenin mixtures, containing both free and glycosidal sapogenins, were dissolved in chloroform–methanol (3:1, v/v) and spotted on Kiesel gel G plates (0.25 mm, 20×20 cm). These were developed with benzene–ethyl acetate–acetic acid (90:8:2, v/v, for the analysis of diosgenin) or with chloroform–acetone–acetic acid (80:20:5, v/v, for yonogenin, tokorogenin and isodiotigenin). The thin-layer plates were freed from the solvents and sprayed with water. Sapogenins were detected as white spots on the light gray background. The areas of Kiesel gel containing diosgenin were scraped off the plates into test tubes and warmed three times with 3 ml of chloroform at 60° for 15 min. The chloroform solutions were filtered and evaporated to dryness. Diosgenin thus extracted was warmed with 0.5 ml of conc. $H_2SO_4$ and 5 ml of $H_3PO_4$ containing 8 mg of $FeCl_3$ for 9 min at 70° and the absorbance of the reaction mixtures were measured at 485 m$\mu$[15] after cooling. The spots of the other sapogenins were scraped into centrifuge tubes and warmed in a boiling water bath with 0.1 ml of ethanol containing 0.5 mg of anisaldehyde for 15 min, then 5 ml of $H_3PO_4$ were added and the solutions again warmed in a boiling water for 70 min. The reaction mixtures were centrifuged at 3500 rpm for 10 min after cooling and the absorbances of the clear supernatants were measured at 540 m$\mu$.

## Results and Discussion

The aerial parts of *D. tokoro* MAKINO showed a pronounced response to the increased day length even though the intensity of the supplementary light was low. The weights of the leaves and stems were significantly increased by the elongation of the illumination period, but the weights of the rhizomes were not affected by this treatment (Table I). The effect of light on the steroidal sapogenins was also obvious in the aerial parts, while the rhizomes were effected very little (Table II). The concentration of yonogenin in the leaves of the long day group was significantly higher than that of the short day group. This difference is better seen by com-

12) A. Akahori, *Phytochemistry*, **4**, 97 (1965); A. Akahori, F. Yasuda and T. Okanishi, *Chem. Pharm. Bull.* (Tokyo), **16**, 498 (1968).
13) G. Blunden, C.J. Briggs and R. Hardman, *Phytochemistry*, **7**, 453 (1968).
14) T. Okanishi and M. Togami, *Chem. Pharm. Bull.* (Tokyo), **17**, 315 (1969).
15) A. Akahori, K. Murata, F. Yasuda, S. Nagase, M. Togami and T. Okanishi, *Ann. Rep. Shionogi Res. Lab.*, **16**, 74 (1966).

Table I. Effect of Day Length on Weight and Water Content of Leaf, Stem and Rhizome of *D. tokoro*

| | Long day (9)[a] | | | Short day (10) | | |
|---|---|---|---|---|---|---|
| | Wet weight (g) | Dried weight (g) | Water content (%) | Wet weight (g) | Dried weight (g) | Water content (%) |
| Leaf | 0.96[b] ±0.11 | 0.18 ±0.02 | 81.2 ± 0.5 | 0.51[c] ±0.05 | 0.11[d] ±0.01 | 78.0[c] ± 0.5 |
| Stem | 0.49 ±0.07 | 0.09 ±0.01 | 81.4 ± 0.6 | 0.23[c] ±0.02 | 0.05[d] ±0.01 | 80.3 ± 0.7 |
| Rhizome | 0.58 ±0.05 | 0.16 ±0.02 | 72.4 ± 1.1 | 0.59 ±0.05 | 0.16 ±0.02 | 72.8 ± 1.0 |

*a*) Figures in parentheses mean the number of the samples.
*b*) mean ± standard error *c*) significant at 1% level *d*) at 5% level

paring the amount of this sapogenin contained in each plant of two the groups. Free isodiotigenin of the leaves responded to light in a different way. Though the concentration of this sapogenin was lower in the long day group than in the short day group, the smaller overall weights of leaves in the latter group meant that there was no significant difference in isodiotigenin content of the two groups. Tokorogenin and the glycosidal isodiotigenin of the leaves was not influenced significantly. A similar tendency to that found for the sapogenins of the leaves was also observed in the stems, but no significant differences were observed except in the glycosidal yonogenin and tokorogenin. The amount of diosgenin in the leaves and stems was too small to be measured by our method. In contrast to the aerial parts; excetp for tokorogenin, the sapogenin content of the rhizome did not show any significant response to the light. The amount of free sapogenins and glycosidal yonogenin and isodiotigenin were below the limit detectable by the analysis.

The increase of yonogenin and the decrease of isodiotigenin caused by the elongation of the light exposure was accompanied by an increase in the growth of the aerial parts. This behaviour of these two sapogenins was similar to that of the sapogenins of the immature *D. tokoro*. As reported previously,[9] isodiotigenin was not found in the seeds of this plant, but appeared first just after germination and gradually increased in amount until the end of the first growing year. The increase of yonogenin appeared a little later than that of isodiotigenin, and at the end of first year the amount of the former slightly exceeded that of the latter. Although the exact quantitative analysis of the sapogenins of this plant has not yet been carried out after its first growth year, isodiotigenin is assumed to gradually decrease in quantity as the plant ages. This sapogenin was also detected in the plants which developed from the section of the rhizome that lacked apical buds.[1] In these plants the growth was distinctly suppressed compared with that of intact plants. Therefore, whether this increase of yonogenin is primarily due to the effect of elongating the day light period or is secondary to the enhancement of the growth, cannot be determined. The amount of diosgenin in the aerial parts was not increased by day length variation. The difference in concentration of diosgenin between the aerial parts of the plants examined by us and by Blunden, *et al.*[13] would probably be not caused by the difference of the day length, although the effects of much more intense light have not yet been examined. According to Garner and Allard,[4] the weights of the aerial parts of *D. divaricata* L. and *D. alata* L. were reduced under the shortened day length, while the tubers of these plants responded variously. Allard[16] further reported that aerial bulbils of *D. opposita* Thunb. were much more freely produced in plants grown under short-day conditions than those illuminated for more than twelve hours and weights of underground tubers were increased

16) H,A. Allard, *Castanea*, **10**, 8 (1945); *idem*, *ibid.*, **12**, 88 (1947); D.G. Coursey, "Yams" Longmans, 1967, p. 42.

Table II. Effect of Day Length on Sapogenins of *D. tokoro*

A. Free Sapogenins

| Part | Day length | Number | Diosgenin (%) | Diosgenin amount per plant (mg) | Yonogenin (%) | Yonogenin amount per plant (mg) | Isodiotigenin (%) | Isodiotigenin amount per plant (mg) | Tokorogenin (%) | Tokorogenin amount per plant (mg) |
|---|---|---|---|---|---|---|---|---|---|---|
| Leaf | L | 9 | — | — | 2.031[c] ±0.141 | 3.788[c] ±0.525 | 0.856[c] ±0.091 | 1.516 ±0.240 | 0.062 ±0.016 | 0.126 ±0.040 |
| | S | 10 | — | — | 0.848 ±0.218 | 1.042 ±0.352 | 1.640 ±0.196 | 1.701 ±0.278 | 0.045 ±0.015 | 0.055 ±0.024 |
| Stem | L | 9 | — | — | 0.100 ±0.020 | 0.089 ±0.017 | 0.119 ±0.030 | 0.090 ±0.010 | 0.010 ±0.003 | 0.011 ±0.005 |
| | S | 10 | — | — | 0.138 ±0.052 | 0.068 ±0.030 | 0.187 ±0.032 | 0.073 ±0.007 | 0.004 ±0.002 | 0.003 ±0.002 |
| Rhizome | L | 9 | — | — | — | — | — | — | — | — |
| | S | 10 | — | — | — | — | — | — | — | — |

B. Saponin-type Sapogenins

| Part | Day length | Number | Diosgenin (%) | Diosgenin amount per plant (mg) | Yonogenin (%) | Yonogenin amount per plant (mg) | Isodiotigenin (%) | Isodiotigenin amount per plant (mg) | Tokorogenin (%) | Tokorogenin amount per plant (mg) |
|---|---|---|---|---|---|---|---|---|---|---|
| Leaf | L | 9 | — | — | 0.564[c] ±0.082 | 0.924[c] ±0.130 | 0.845 ±0.136 | 1.338 ±0.164 | 0.093 ±0.013 | 0.160 ±0.029 |
| | S | 10 | — | — | 0.121 ±0.029 | 0.135 ±0.032 | 0.934 ±0.108 | 1.007 ±0.146 | 0.069 ±0.011 | 0.079 ±0.015 |
| Stem | L | 9 | — | — | 0.077 ±0.014 | 0.061[d] ±0.007 | 0.147 ±0.029 | 0.112 ±0.017 | 0.176 ±0.038 | 0.130[c] ±0.019 |
| | S | 10 | — | — | 0.077 ±0.010 | 0.038 ±0.007 | 0.196 ±0.020 | 0.082 ±0.006 | 0.152 ±0.015 | 0.067 ±0.008 |
| Rhizome | L | 9 | 0.220 ±0.015 | 0.357 ±0.048 | — | — | — | — | 0.280[c] ±0.008 | 0.450 ±0.044 |
| | S | 10 | 0.243 ±0.006 | 0.378 ±0.047 | — | — | — | — | 0.424 ±0.046 | 0.622 ±0.068 |

C. Total Sapogenins

| Part | Day length | Number | Diosgenin (%) | Diosgenin amount per plant (mg) | Yonogenin (%) | Yonogenin amount per plant (mg) | Isodiotigenin (%) | Isodiotigenin amount per plant (mg) | Tokorogenin (%) | Tokorogenin amount per plant (gm) |
|---|---|---|---|---|---|---|---|---|---|---|
| Leaf | L | 9 | — | — | 2.596[c] ±0.176 | 4.712[c] ±0.581 | 1.701[d] ±0.208 | 2.854 ±0.356 | 0.155 ±0.026 | 0.287[c] 0.064 |
| | S | 10 | — | — | 0.970 ±0.226 | 1.077 ±0.036 | 2.573 ±0.222 | 2.708 ±0.230 | 0.114 ±0.025 | 0.134 ±0.040 |
| Stem | L | 9 | — | — | 0.177 ±0.028 | 0.150 ±0.020 | 0.266 ±0.048 | 0.202[c] ±0.017 | 0.186 ±0.037 | 0.141[c] ±0.019 |
| | S | 10 | — | — | 0.214 ±0.060 | 0.112 ±0.038 | 0.385 ±0.053 | 0.154 ±0.011 | 0.162 ±0.017 | 0.071 ±0.009 |

under short-day conditions. Similar results were obtained with Nigerian species,[17] that is, the development of tubers was enhanced by short-day condition. In our present experiment too, variation of the day length did not affect the weights of rhizomes. Bennett, *et al.*[18] postulated that the steroidal sapogenins are synthesized in the aerial parts and rather rapidly translocated to the tuber. However, the present data on the difference in behaviour of the sapogenins suggest that this rapid translocation may not occur.

17) E. Njoku, *J. West Afr. Sci. Ass.*, **8**, 29 (1963); D.G. Coursey, "Yams" Longmans, 1967, p. 42.
18) R.D. Bennett, E. Heftmann and W.H. Preston, Jr., *Arch. Biochem. Biophys.*, **103**, 74 (1963).