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STUDIES ON THE STEROIDAL COMPONENTS OF DOMESTIC PLANTS—LVI.*

CHANGES IN THE SAPOGENIN COMPOSITION OF *DIOSCOREA TOKORO* IN ITS FIRST SEASON'S GROWTH FROM SEED

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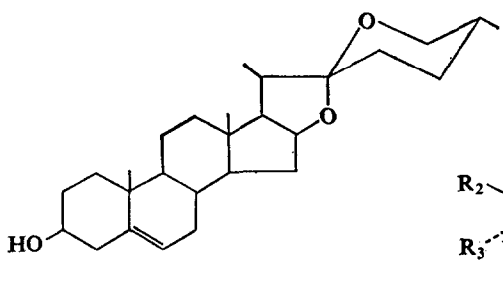
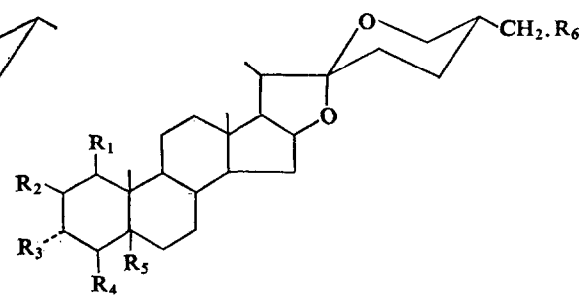
Abstract—A marked change in the steroidal sapogenins due to the age of the plant was found in seedlings of *Dioscorea tokoro* Makino, when the whole plant was analysed up to age of 165 days. Isodiotigenin first appeared in the seedlings 10 days after germination and increased in quantity successively thereafter. The change in the amounts of yonogenin and isodiotigenin was discussed as compared with the behaviour of their 25L-isomers, neoyonogenin and diotigenin in the *D. tenuipes* complex.

INTRODUCTION

THE STEROIDAL sapogenins of mature plants of Japanese *Dioscorea* species were investigated by one of the authors (A. A.).[1–4] He isolated yonogenin (II) (25D,5β-spirostane-2β,3α-diol) and tokorogenin (III) (25D,5β-spirostane-1β,2β,3α-triol) from the aerial parts of *D. tokoro*



Diosgenin (I)



R_1,R_4,R_5,R_6 = H; R_2,R_3 = OH Yonogenin (II)
R_4,R_5,R_6 = H; R_1,R_2,R_3 = OH Tokorogenin (III)
R_1,R_5,R_6 = H; R_2,R_3,R_4 = OH Isodiotigenin (IV)
R_4,R_6 = H; R_1,R_2,R_3,R_5 = OH Kogagenin (V)
R_1,R_4,R_5 = H; R_2,R_3,R_6 = OH Igagenin (VI)

* Part LV. AKIRA AKAHORI, ISAMU OKUNO, TAMETO OKANISHI and TORU IWAO, *Chem. Pharm. Bull.*, in press.

[1] A. AKAHORI, *Ann. Rep. Shionogi Res. Lab.* **11**, 93 (1961).
[2] A. AKAHORI, *Ann. Rep. Shionogi Res. Lab.* **11**, 97 (1961).
[3] A. AKAHORI, *Ann. Rep. Shionogi Res. Lab.* **13**, 68 (1963).
[4] A. AKAHORI, *Phytochem.* **4**, 97 (1965).

Makino and detected diosgenin (I) (25D-spirost-5-en-3β-ol) and kogagenin (V) (25D-spirostane-1β,2β,3α,5β-tetraol) in these parts by paper chromatographic analysis. From the underground parts of this plant, diosgenin was isolated in addition to these sapogenins. He also examined the free sapogenins of the aerial parts of this plant collected from the different

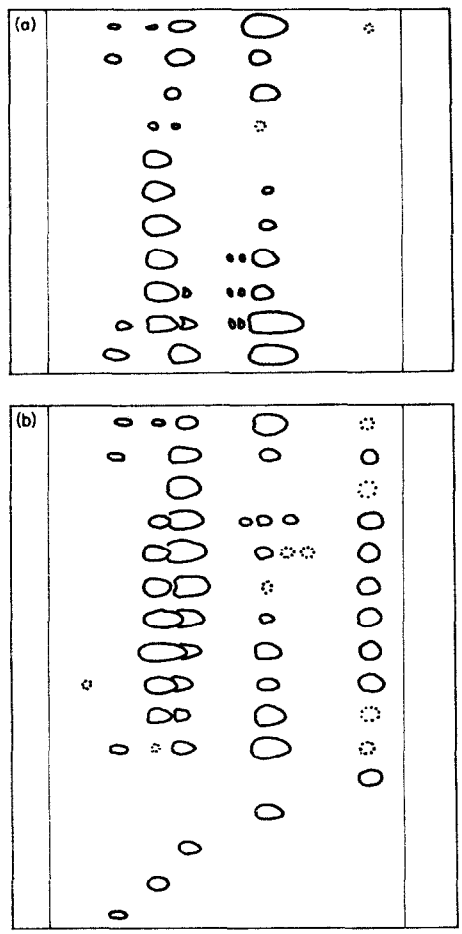


FIG. 1. PAPER CHROMATOGRAMS OF THE SAPOGENINS OF *Dioscorea tokoro*.

(*a*) Free sapogenins. Female flower. Dormant seed. 10 days' seedling, 20 days' seedling, 30 days' seedling, 40 days' seedling, 72 days' seedling, 103 days' seedling, 165 days' seedling. Leaf of 2nd-year plant. Leaf of 3rd-year plant.

(*b*) Saponin-type sapogenins. Female flower. Dormant seed. 10 days' seedling, 20 days' seedling, 30 days' seedling, 40 days' seedling, 72 days' seedling, 103 days' seedling, 165 days' seedling. Leaf of 2nd-year plant. Leaf of 3rd-year plant. Diosgenin, Yonogenin, Tokorogenin, Isodiotigenin, Koganenin.

parts of Japan and always detected yonogenin, tokorogenin and kogagenin by paper chromatography (in contrast to the wide variation found in the sapogenins of *D. tenuipes* complex[5]) and supposed that the sapogenin composition of *D. tokoro* did not change qualitatively throughout its life. This led to the conclusion that *D. tokoro* lacked diotigenin

[5] A. AKAHORI, *Acta. Phytotax. Geobot.* **21**, 149 (1965).

(25L, 5β-spirostane,2β,3α,4β-triol) and for him to adopt this chemical for distinguishing between *D. tokoro* and the *D. tenuipes* complex.[4,5] However, the authors recently isolated

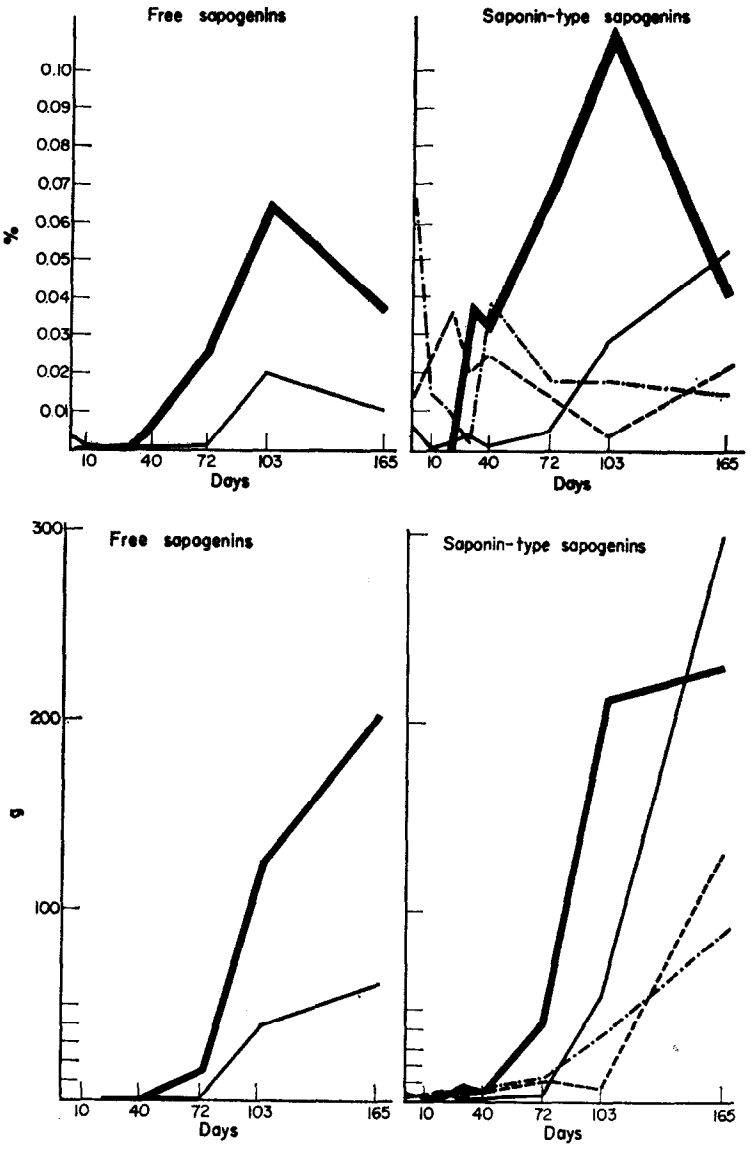


FIG. 2. VARIATIONS IN THE SAPOGENINS OF *D. tokoro* SEEDLINGS WITH AGE.

(*a*) Sapogenin concentration: isodiotigenin ▬, yonogenin ——, diosgenin – – –, tokorogenin –·–·–.

(*b*) Amounts of sapogenins per plant: isodiotigenin ▬, yonogenin ——, diosgenin – – –, tokorogenin –·–·–.

igagenin (VI) (25D,5β-spirostane-2β,3α,27-triol) from the female flowers of this plant.[6] This sapogenin was not found in the other parts which suggested that the sapogenin composition

[6] A. AKAHORI, I. OKUNO, T. OKANISHI and T. IWAO, *Chem. Pharm. Bull.*, in press.

might also change within the plant. The present work was undertaken to elucidate the change in the steroidal sapogenins of this plant due to its age.

RESULTS

Paper chromatograms of the sapogenins of seedlings, female flowers and seeds are illustrated in Fig. 1. To characterize the spots observed, 1170 mg of the hydrolysis products of whole plants, harvested 165 days after the seed had been placed on moistened filter papers, were chromatographed on alumina (Merck) and the following sapogenins were isolated as crystals: (1) 8 mg diosgenin (I), m.p. 194–196°; (2) 5 mg yonogenin (II), m.p. 238–240°; (3) 9 mg tokorogenin (III), m.p. 262–266°; (4) 49 mg white platelets, m.p. 281–282°, $[\alpha]_D^{22} - 53{\cdot}9°$ ($c = 0{\cdot}382$, MeOH), $C_{27}H_{44}O_5$, R_f 0·28, and (5) 0·5 mg of white powder, R_f 0·12. (4) was identified as isodiotigenin (IV) (25D,5β-spirostane-2β,3α,4β-triol) by comparing its m.p., i.r. and mass spectra with those of an authentic sample. (5) was difficult to crystallize and was refluxed in acetone containing 0·1 % *p*-toluenesulfonic acid. An acetonide was thus obtained as white platelets, m.p. 298–300°, $[\alpha]_D^{22} - 26{\cdot}2°$ ($c = 0{\cdot}263$, $CHCl_3$), $C_{31}H_{48}O_6$. This was identified as the monoacetonide of isotenuipegenin, derived from tenuipegenin,[7] by m.p., i.r. and mass spectra. It was confirmed that the spots with R_f values 0·91, 0·64, 0·37, 0·28 and 0·17 corresponded to those of diosgenin, yonogenin, tokorogenin, isodiotigenin and isotenuipegenin, respectively. The spot of kogagenin was not detected in the paper chromatograms of the first-year plants. Changes in the concentrations of individual sapogenins with age of the plant are summarized in Fig. 2.

DISCUSSION

Changes of the steroidal sapogenins due to the age of the plants have been discussed by several authors.[8–12] Marker *et al.*[8] found that the predominant sapogenins isolated from young, mature, old and flowering plants of *Agave* have successively fewer hydroxyl groups. In the case of *Yucca*, which does not die after flowering unlike *Agave*, they isolated complex mixtures of sapogenins from the plants before flowering and mixtures of fewer sapogenins or only one sapogenin possessing fewer hydroxyl groups from the plants at the flowering period. They postulated that the polyhydroxy-Δ^5-sapogenins are synthesized at first in the plants and converted to monohydroxysapogenins following enzymic reduction of the 5,6-double bond and dehydration. Dadiwar and Fayes[9] recognized the same tendency among the sapogenins of *Agave sisalana* Perinne. However, they found the reappearance of gitogenin in the flowering stalk of this plant and considered it a result of the mechanism by which the plant dies away and then prepares to give a new life. The change of the constitution of the steroidal sapogenins due to age is also found in *Dioscorea tokoro*. Although yonogenin, tokorogenin and kogagenin are contained in aerial parts of mature plants of this species together with a small amount of diosgenin, regardless of the site and season, a 27-hydroxysapogenin, igagenin,[13]

[7] A. AKAHORI, F. YASUDA and T. OKANISHI, *Chem. Pharm. Bull.* **16**, 499 (1968); Tenuipegenin is a 25L-tetrahydroxysapogenin, which yields a tetracetate and mono- and diacetonide by mild acetylation and treatment with acetone and *p*-toluenesulfonic acid respectively. It structure is now being investigated.

[8] R. E. MARKER *et al.*, *J. Am. Chem. Soc.* **69**, 2167 (1947).

[9] A. A. DADIWAR and M. B. E. FAYES, *Arch. Biochem. Biophys.* **92**, 420 (1961).

[10] A. M. WOODBURY, M. E. WALL and J. J. WILLAMAN, *Econ. Botany* **15**, 79 (1961).

[11] M. E. WALL, B. H. WARNOCK and J. J. WILLAMAN, *Econ. Botany* **16**, 266 (1962).

[12] E. A. BAKER, J. T. MARTIN and A. P. WILSON, *Ann. Appl. Biol.* **58**, 203 (1966).

[13] F. YASUDA, Y. NAKAGAWA, A. AKAHORI and T. OKANISHI, *Tetrahedron*, in press.

is found only in the female flowers and disappears as the flowers die. The concentration of the sapogenin is very much lower in the dormant seeds than in the flowers. In the seeds, diosgenin, yonogenin, tokorogenin and kogagenin are present while igagenin is not found.[6] When the seeds are planted, a marked change occurs. The decrease in concentration of sapogenins from day 0 to day 10 is partly due to the increase in weight of the seeds by the absorption of water. The content of the ether extracts decreases up to 40 days but this decrease is due to the decrease of an oily substance, because the ether extracts of the seedlings immediately after germination are chiefly composed of oily substances. The concentrations of the free sapogenins of the seedlings do not change until 20 days. Then a new sapogenin, isodiotigenin, appears, which rapidly increases in amount and becomes the predominant sapogenin. The increase of yonogenin occurs a little later. The decrease of the concentration of isodiotigenin and yonogenin at the end of the first year and the rather constant concentrations of diosgenin and tokorogenin through the first year are due to the marked growth of the seedlings and the total amounts of these four sapogenins contained in individual seedlings increase successively. Isodiotigenin is also found in the leaves of the second-year plants but its detection becomes almost impossible in third-year plants. Although it is not clear whether total amounts of isodiotigenin contained in individual plants actually decrease in the second and third year or not, as the quantitative investigation of the sapogenins of this plant is not yet accomplished, it is quite probable that this sapogenin is synthesized only at an earlier stage or it is rapidly converted to other sapogenins in the older plants. Baker *et al.*[12] suggested the possibility of the synthesis of diosgenin at the actively growing parts of the shoot and the translocation of this sapogenin to the rhizome in their study on *D. deltoidea* Wall. However, changes in the ratio of diosgenin to yonogenin between the aerial and underground parts of *D. tokoro*, and the appearance and disappearance of isodiotigenin cannot be so easily explained. Unlike yonogenin and isodiotigenin in *D. tokoro*, diotigenin, a 25L-epimer of isodiotigenin is a predominant sapogenin contained in the aerial parts of the Rokko population of *D. tenuipes* complex and neoyonogenin, a 25L-epimer of yonogenin is contained in negligible amount in this plant.[4,7] On the other hand, tokorogenin and/or its 25L-epimer neotokorogenin are contained in considerable amount in both plants, even in *D. tenuipes* collected in eastern Japan which contains neither diotigenin nor neoyonogenin. These facts suggest that yonogenin and isodiotigenin are interconvertible and tokorogenin is synthesized by another pathway. Furthermore, the increase of yonogenin in the seedlings of *D. tokoro*, which follows the increase of isodiotigenin, supports Marker's theory at least for the relation between yonogenin and isodiotigenin.

Because the climatic data recorded between April and October, 1967, at Aburahi Farm did not greatly deviate from the average of that during the past 16 years, it is estimated that this marked change of the steroidal sapogenins normally occurs during the development of *D. tokoro* in the field even though some parts of this change may be due to climatic factors.

EXPERIMENTAL

Seeds

Seeds were collected in October 1966 at and around Aburahi Farm, Shiga Pref. and stored in a desiccator until April 1967.

Germination and Cultivation

The seeds were placed on moistened filter papers on 20 April 1967 (day 0) and kept in the dark at 20°. The seedlings germinated between 1 and 4 May were transplanted to the field on 4 May. Vine supports were not constructed.

1700 seeds, germinated between 9·00 on 29 April and 15.00 on 30 April, were collected, gently pressed in filter papers to free them of excess water, weighed and immediately immersed in methanol. The seedlings growing in the field were harvested on 10, 20 and 30 May, 1 July, 1 August and 2 October, washed with water and treated as above.

The leaves of the second- and third-year plants, which were sown in the field April 1965, were harvested 1 August 1966 and 16 August 1967 respectively and dried at 70°.

Extraction

The seedlings were homogenized in methanol and extracted with methanol (×3) under reflux. The methanol extracts were combined, evaporated and again extracted with ether under reflux. The ether extracts were examined by paper chromatography for free sapogenins. The residues, after extraction with ether, were hydrolysed in methanol containing 5% HCl under reflux. The hydrolysis products were extracted into ether.

The dormant seeds and the dried leaves of the second- and third-year plants were extracted with CH_2Cl_2 and then with methanol in a Soxhlet. The amethanol extracts were hydrolysed as above.

Paper Chromatography

The extracts were dissolved in methanol, spotted on Toyo-Roshi, No. 50 (2 × 40 cm), and developed with toluene–acetic acid (50:3). The paper were air-dried, sprayed with 1% cinnamic aldehyde in ethanol, heated, sprayed with 25 g $SbCl_3$ in 5 ml nitrobenzene and again heated.[14] The spots of the steroidal sapogenins were detected as yellow to orange spots. R_f values were: diosgenin, 0·91; yonogenin, 0·64; tokorogenin, 0·36; isodiotigenin, 0·26; kogagenin, 0·18.

Quantitative Procedure

Aliquots of the ether extracts and hydrolysis products were analysed according to the method of Okanishi and Togami.[15] The samples were spotted on thin-layer plates (20 × 20 cm, Silica gel G, 250μ) and developed with chloroform–acetone–acetic acid (80:20:5). The spots of the steroidal sapogenins were detected as yellowish brown spots in the chambers saturated with iodine vapour and marked. The marked spots corresponding to authentic yonogenin, tokorogenin and isodiotigenin were transferred to test tubes, anisaldehyde and H_3PO_4 were added and the tubes were warmed in a water bath for 70 min. The test tubes were then centrifuged at 3500 rev/min, for 10 min, cooled, and the absorbances of the clear supernatant were measured at 540 nm with a Hitachi Photo-electric Spectrophotometer, Model EPU-2A. Diosgenin was extracted with chloroform from silica gel, then developed with $FeCl_3$ and H_3PO_4–H_2SO_4 (10:1, v/v) and the absorbance was measured at 485 nm.

[14] T. OKANISHI, A. AKAHORI and F. YASUDA, *Ann. Rept. Shionogi Res. Lab.* **8**, 927 (1958).
[15] T. OKANISHI and M. TOGAMI, *Chem. Pharm. Bull.*, in press.