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Isolation and Characterization of Steroidal Sapogenins and Glycoalkaloids from Tissue Cultures of *Solanum verbascifolium* Linn.

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Undifferentiated callus tissue of *S. verbascifolium* Linn. was established from sterilized seeds on Murashige and Skoog's revised medium and maintained on the same medium. Six-month-old callus was then analyzed for steroidal sapogenins and glycoalkaloids. Diosgenin and solasodine were isolated and estimated quantitatively in tissue samples harvested periodically (2, 4, 6 and 8 weeks).

Keywords——*Solanum verbascifolium*; Solanaceae; tissue cultures; diosgenin; solasodine; *in vitro*

Steroidal sapogenins and glycoalkaloids have been reported to occur in tissue cultures of a number of *Solanum* species.[1-7] However, there is no indication in the literature of the presence of diosgenin and solasodine, which are pharmaceutically important compounds, in tissue cultures of *S. verbascifolium*, although the leaves, stem and fruits of this plant species have been analyzed[8] for steroidal sapogenins and/or glycoalkaloids. Here we report the isolation and characterization of diosgenin and solasodine from cell cultures of this species.

Experimental

Unorganized static cultures of *S. verbascifolium* were established from the seeds[9] on Murashige and Skoog's revised medium (RT) supplemented with 1 ppm of 2,4-dichlorophenoxyacetic acid (2,4-D) and 1% agar.[10,11] The static cultures were allowed to grow for a period of six months with frequent subculturings (6—8 weeks) in fresh RT medium at $26 \pm 1°$ in room light.

Tissues were harvested at transfer ages of 2, 4, 6 and 8 weeks, then dried and the growth index was calculated in each case (GI = final dry weight of tissue-initial dry weight of tissue/initial dry weight of tissue). All the dried tissue samples were separately analyzed for sapogenins and glycoalkaloids. Five replicates of each sample were examined and the data were averaged (SE<0.05—0.06%).

Extraction and Separation——Steroidal Sapogenins: Each tissue sample was powdered, hydrolyzed with 5% (w/v) HCl in ethanol for 4 hr[3] and filtered. The filtrate was partitioned with ethyl acetate and the resulting upper layer was washed with distilled water till neutrality (pH 7.0). Each fraction was dried *in vacuo*, dissolved in chloroform and analyzed further.[12]

Thin-layer chromatography was carried out on activated plates of silica gel G (BDH) (wet thickness 250 μm). Various extracts along with standard compounds (diosgenin, gitogenin, hecogenin, smilagenin, tigogenin and yamogenin) dissolved in chloroform were spotted on the plates and developed with benzene–ethyl acetate (3:2)[7] or hexane–ethyl acetate (3:1).[13] The plates were sprayed with 50% sulfuric acid[14] and with anisaldehyde reagent[13] separately. These plates were then heated at 100° for 10 min. A spot (*Rf* 0.57, pink) corresponding to the reference diosgenin was observed in each sample. This spot was collected by preparative TLC using benzene–ethyl acetate (3:2), eluted with chloroform and concentrated to dryness. The isolated compound was crystallized from methanol–acetone: mp[15] (204—206°), mmp (undepressed), $[\alpha]_D^{25} -129°$ and IR spectrum (984, 922, 901 and 866 cm^{-1}). The values were in accordance with those reported for authentic diosgenin.[13]

Diosgenin was estimated quantitatively (μg/gram dry weight) in all the tissue samples by the spectrophotometric method of Sanchez *et al.*[16]

Steroidal Glycoalkaloids: Another part of each of the powdered tissue samples was extracted with 2% acetic acid in 90% ethanol for 24 hr. The extract thus obtained was concentrated, mixed with an equal volume of 10% acetic acid and filtered.[17] The filtrate was made alkaline with ammonia (pH 9—10). A dark brown precipitate was formed in each case, and was dissolved in hot methanol. The solution was filtered and the filtrate was dried *in vacuo* (crude glycoalkaloid). A part of the crude glycoalkaloid from each sample was hydrolyzed (2 ml HCl: 10 ml ethanol).[6] Needle-like crystals of the aglycone hydrochloride were ob-

tained, and these were heated with ammonia solution for 1 hr on a water bath. The free aglycone thus liberated was extracted with chloroform and then crystallized from 80% methanol.

The aglycone fraction of each sample was taken up in chloroform and applied to silica gel G coated and activated plates along with authentic solasodine and solanidine. The plates were developed in chloroform and methanol (19: 1) and sprayed with Dragendorff's reagent. A single positive spot (*Rf* 0.24) corresponding to that of the standard solasodine was observed in each case.

The isolated compound showed mp (199—200°), mmp (undepressed), $[\alpha]_D^{25}-97.1$, and its IR spectrum coincided with that of the reference sample of solasodine. Solasodine was estimated quantitatively in all the tissue samples following the method of Birner.[18]

Results

Undifferentiated callus was greyish-brown in color and fragile in nature. The growth index was maximun (6.6) in six-week-old tissue. It had declined (4.6) by the eighth week, but was still higher than that at the fourth week (4.2).

Diosgenin content was much higher (146.6 μg/g.d.w.) in six-week-old tissue with a decrease in the eighth week (128.5 μg/g.d.w.) to a level even below that at two weeks (133.1 μg/g.d.w.). However, solasodine content was maximum (54.0 μg/g.d.w.) at the eighth week (Table 1), suggesting that there is some relation between their yields.

TABLE I. Production of Diosgenin and Solasodine by Tissue Cultures of *Solanum verbascifolium* LINN.

Age of cultures (weeks)	Growth index[a]	Diosgenin μg/gdw[b]	Solasodine μg/gdw
2	2.6	133.1	23.2
4	4.2	136.2	40.0
6	6.6	146.6	46.6
8	4.6	128.5	54.0

a) Growth index = final dry weight of tissue − initial dry weight of tissue/initial dry weight of tissue.
b) μg/gdw = μg/gram dry weight.

Doepke *et al.*[19] reported on steroid sapogenin and alkaloid contents in crude extracts of different plant parts of *S. verbascifolium* and found maximum contents of 50% and 8.5—10.5% of solasodine and diosgenin respectively. However, this is the first time that work on the identification and contents of diosgenin and solasodine in tissue culture of *S. verbascifolium* has been carried out.

It is evident that cell cultures of this plant species have the potential to synthesize diosgenin and solasodine, but the amounts are lower than in the intact plant parts. Solasodine is produced in much lower amounts (54.0 μg/g.d.w.) than diosgenin (146.6 μg/g.d.w.).

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The Decomposition of Tryptophan in Acid Solutions: Specific Effect of Hydrochloric Acid

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The stability of tryptophan in aqueous solutions of HCl, H_2SO_4, or CH_3SO_3H under aerobic conditions was examined, and a specific effect of HCl was found. A kinetic study of the HCl-induced decomposition, tests for the free chlorine formation on heating the HCl used for the above run, *etc.* suggested that free chlorine produced by the air oxidation of HCl may have participated in the decomposition of tryptophan.

Thin-layer and ion-exchange chromatography of the decomposition products of tryptophan revealed that oxindolylalanine and dioxindolylalanine were formed by the reaction. These two compounds were also formed by treating tryptophan with ClO^- or N-chlorosuccinimide in a solution of HCl. These results support the above possibility.

Keywords——tryptophan; hydrochloric acid; free chlorine; oxindolylalanine; dioxindolylalanine

The stability of tryptophan in hot 6 N HCl has been investigated in connection with the decomposition of tryptophan during acid hydrolysis of protein. These studies[1] revealed that tryptophan was relatively stable when heated under anaerobic conditions. However, there are few papers dealing with the decomposition of tryptophan on being heated in a solution of HCl under aerobic conditions, *e.g.*, factors influencing the decomposition, the decomposition product, the mechanism involved, *etc.* In addition, it is not clear whether tryptophan is decomposed similarly when heated in other acid solutions.

In the present investigation, we examined the stability of tryptophan on being heated in solutions of HCl, H_2SO_4 or CH_3SO_3H (MSA) under aerobic conditions, and found that tryptophan was decomposed specifically in HCl solution. On the basis of this finding, further work was carried out to clarify the mode of action of HCl on tryptophan.

Experimental

Chemicals——L-Tryptophan was obtained from Kanto Kagaku Co. (Tokyo). DL-Oxindolylalanine (2,3-dihydro-2-oxo-tryptophan) was prepared by the method of Wieland *et al.*[2] and dioxindolylalanine (2,3-dihydro-3-hydroxy-2-oxo-tryptophan) by the method of Savige.[3] These amino acids were confirmed to be chromatographically homogeneous by means of an amino acid analyzer. Constant-boiling HCl (here-