SOLANUM JASMINOIDES PAXT., TISSUE CULTURES. I. PRODUCTION OF STEROIDAL SAPOGENINS AND GLYCOALKALOIDS.[1]

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Solanum jasminoides Paxt., was investigated for glycoalkaloids (1, 2) and steroids (3) in different plant parts. In a continuation of our earlier findings (1–3), we now report the isolation and identification of diosgenin and solasodine in callus culture of *Solanum jasminoides* Paxt.

EXPERIMENTAL

PLANT MATERIAL AND ESTABLISHMENT OF TISSUE CULTURE.—The plants of *S. jasminoides*, from which the callus cultures originated, were collected from the Botanical Garden, University Maharaja's College, Jaipur, India, during March 1978; the stem pieces with buds were used. A voucher specimen has been deposited in the herbarium of the Department of Botany, University of Rajasthan, Jaipur, India.

Unorganized callus tissue cultures of the plant were established from the sterilized stem pieces with buds on Revised Murashige and Skoog's medium (RT) supplemented with 1 ppm of 2,4-dichlorophenoxyacetic acid (2,4-D) and 1% agar (4, 5). The tissue was maintained for 14 months by subculturing every 6 to 8 weeks to fresh RT medium. The tissue thus maintained was then transferred onto fresh RT medium, harvested at 2, 4, 6 and 8 weeks and analyzed for steroids and glycoalkaloids. Each tissue sample was dried in an oven at 100° for 15 min so as to inactivate the enzymatic activity and then at 60° till a constant weight was achieved. The growth indices (GI) were calculated separately for each harvest (final dry weight of tissue—initial dry weight of tissue/initial dry weight of tissue).

EXTRACTION PROCEDURE AND ANALYSIS.—*STEROIDAL SAPOGENINS:* The tissue thus harvested was dried and powdered. Each powdered sample was hydrolyzed with 5% (w/v) HCl in ethanol for 4 hr. The filtrate was extracted with ethyl acetate three times and the ethyl acetate fraction was washed to neutrality with distilled water, dried and reconstituted in chloroform for further analysis (6, 11).

Each crude extract was separately spotted along with the reference sapogenins on air-dried silica gel G (wet thickness 250 mμ) plates and developed with benzene-ethyl acetate (3:2) as the solvent system. The steroidal compounds were located by spraying with 50% sulfuric acid or with anisaldehyde reagent (3), and heating at 100° until the characteristic colors developed (7). The spot corresponding to reference diosgenin was separated by preparative tlc (silica gel G; wet thickness 500 mμ; benzene-ethyl acetate, 3:2), eluted with chloroform, tested by tlc again for purity and later crystallized from methanol-acetone.

The isolated diosgenin was subjected to mp (203–205°), mmp (204°), acetate mp (193–194°), $[\alpha]^{25}D$ −129°, and ir spectrum (characteristic peaks at 984, 922, 901 and 866 cm^{-1}) studies. These data for the isolated diosgenin were in accord with the values reported for standard diosgenin (3). Quantitative estimation of diosgenin was conducted colorimetrically on a spectrophotometer (Carl Zeiss, Jena DDR, VSU-2P) by the method of Sanchez *et al.* (8).

STEROIDAL GLYCOALKALOIDS. Another part of the dried and powdered tissue of each sample was macerated with 90% ethyl alcohol containing 2% acetic acid at room temperature. The filtrate was concentrated, an equal volume of 10% acetic acid was added, and the mixture allowed to stand overnight in a refrigerator (1, 9). The aglycone fraction from each of the samples thus obtained was dissolved in chloroform and analyzed further.

Each crude extract was separately spotted along with reference compounds on tlc (silica gel G) and developed in chloroform-methanol (19:1). A single Dragendorff's positive spot coinciding with that of the reference solasodine was observed. This corresponding spot was separated by preparative tlc under the above conditions, eluted with chloroform and crystallized from 80% methanol.

The isolated compound was subjected to mp, 199–200°, (lit. (12) 197–198°), mmp (undepressed), $[\alpha]^{25}D$ −97.10 and superimposable ir spectral studies. The data for the isolated solasodine agreed with the values reported for standard solasodine (2, 12).

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Quantitative estimation of solasodine was carried out for each sample following Birner's spectrophotometric method (10).

RESULTS

The undifferentiated tissue was greyish-black in color and fragile in nature. The tissue showed a steady increase in growth index with a maximum (7.0) up to 6 weeks and then decreased by the eighth week (table 1). The diosgenin concentration was maximum in the eighth week (0.099%), whereas the minimum (0.069%) occurred after 2 weeks. Solasodine was, however, maximum (0.19%) in the sixth week, and the minimum was found to be in the eighth week (0.092%). While comparing the presence of diosgenin in the intact plant (3; roots—0.36%; flowers —0.18%; aerial parts—lacking) with the maximum production in tissue culture (0.099%) in the eighth week, it was found that the intact plant system was able to synthesize diosgenin in a greater amount. Similar results were observed in the intact plant (1, 2) for solasodine, where it is produced in higher amounts (roots—0.008%; flowers—0.23%; aerial parts—0.30%) than in the tissue culture system (0.19% in six weeks). It is noteworthy that in the stem pieces, diosgenin production is altogether absent. However, callus cultures derived from stem could biosynthesize diosgenin. Thus, the results suggest that the cell culture system of *S. jasminoides* has an advantage in having a higher potential for synthesizing these metabolites, although in lower amounts, than the intact plant.

TABLE 1. Growth index (GI), diosgenin and solasodine contents in *Solanum jasminoides* Paxt., tissue culture.[a]

Age of culture (weeks)	GI[b]	Diosgenin[c] (%)	Solasodine[c] (%)
2	3.5	0.069	0.100
4	4.8	0.083	0.094
6	7.0	0.067	0.190
8	5.6	0.099	0.092

[a]The data is the mean of five replicates (SE<0.05%.
[b]GI=Growth Index (Final dry weight of tissue—initial dry weight of tissue/Initial dry weight of tissue).
[c]On dry weight basis.

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