Note

Growth and Production of Steroidal Sapogenins and Glycoalkaloids in *Solanum jasminoides* Paxt., Suspension Cultures

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Received June 9, 1981

Solanum jasminoides Paxt., has been investigated for steroidal glycoalkaloids^{1,2)} and steroids *in vivo*³⁾ and *in vitro*.⁴⁾ With an aim to find out the potentialities of callus tissue in suspension cultures, this work which is in continuation of our previous investigations,^{1~4)} deals with the isolation and characterization of diosgenin and solasodine in suspension cultures of *S. jasminoides*.

Establishment of tissue cultures. Callus tissues of *S. jasminoides* were established⁴⁾ from the stem cuttings on Murashige and Skoog's⁵⁾ revised⁶⁾ (RT) medium. These tissues were maintained (for 14 months) onto fresh RT medium by periodic subcultures (6~8 weeks). Such tissues were transferred to RT liquid medium supplemented with 0.1 ppm of 2,4-dichlorophenoxyacetic acid. The cultures were grown on a reciprocal shaker at normal room light conditions. These suspension tissues were maintained for five months by regular subcultures (4~6 weeks). The tissue was then transferred to fresh RT liquid medium and allowed to grow to a period of 2, 4, 6 and 8 weeks. The tissues were harvested at these time intervals, dried and analysed further. Their growth indices were calculated ($GI = \text{Final weight of tissue} - \text{initial weight of tissue} / \text{initial weight of tissue}$).

Isolation and characterization.

Steroidal sapogenins. Each powdered tissue sample was refluxed with 5% (w/v) HCl in EtOH for 4 hr.⁷⁾ The filtrate was partitioned with EtOAc three times and the EtOAc layer was neutralized with distilled water by repeated washings. Each extract was dried and reconstituted in chloroform before analyses.

Different crude extracts were applied separately along with the reference sapogenins on silica gel G(BDH, wet thickness 250 μm) plates and developed⁸⁾ in CHCl_3 -MeOH (99:1) as the solvent system. The steroidal compounds were located either by spraying with 50% H_2SO_4 or anisaldehyde reagent subsequently heating (100°C) until the characteristic colors developed.⁹⁾ The spot corresponding to reference diosgenin (R_f 0.56, Pink) was separated by preparative TLC (silica gel H; wet thickness

500 μm) using the above solvent system and eluted with CHCl_3 . Each eluate was dried and crystallized from methanol.

The isolated diosgenin was confirmed on the basis of co-TLC and color reactions, mp, 204°~205°C, mp, 203°C, $[\alpha]_D^{25} = 129^\circ$ ($c = 0.95$ in CHCl_3) and characteristic IR peaks. These data were in accordance with the values reported for standard diosgenin. Quantitative estimation of diosgenin was, however, conducted colorimetrically using the method of Sanchez *et al.*¹⁰⁾

Steroidal glycoalkaloids. Another half of the powdered tissue of each sample was extracted with 2% AcOH in 90% EtOH for 24 hr. The filtrate was reduced in volume, an equal volume of 10% AcOH was added and left in a refrigerator.^{4,11)} The resulting dark brown ppt was dissolved in hot MeOH, filtered and dried to yield crude glycoalkaloid. On hydrolysis of the part of crude glycoalkaloid, the free aglycone liberated was extracted with CHCl_3 , dried and crystallized from methanol.

The aglycone fraction of each sample was taken up in CHCl_3 and spotted along with reference compounds on TLC (silica gel G). The plates were developed in CHCl_3 -MeOH (19:1), and sprayed with Dragendorff's reagent which showed a spot coinciding to reference solasodine (R_f 0.24). This spot was separated by preparative TLC, eluted with CHCl_3 and crystallized from methanol.

The isolated solasodine was confirmed by mp, 199°~201°C, mp, 200°C, optical rotation and characteristic IR peaks which agreed with the values reported for standard solasodine.¹²⁾ Its quantitative estimation was made using Birner's spectrophotometric method.¹³⁾

The cells in suspension cultures were brown in color. A linear increase in the growth indices, diosgenin and solasodine contents with a maximum (8.24, 0.159, 0.203%, respectively) was observed with the increasing growth period up to 6th week but all these declined during the 8th week (Table I).

While comparing the growth and the yield of these metabolites with the static cultures, there was a significant increase in suspension cultures.

TABLE I. GROWTH INDICES, DIOSGENIN AND SOLASODINE CONTENTS IN *Solanum jasminoides* PAXT., SUSPENSION CULTURES

Age of culture (weeks)	GI ^a	Diosgenin (%)	Solasodine (%)
2	4.10	0.081	0.145
4	6.45	0.122	0.113
6	8.24	0.159	0.203
8	6.04	0.132	0.147

^a Growth index. On dry weight basis. The data is the mean of five replicates.

It is interesting to note that the diosgenin synthesis which was completely absent in stem pieces, from which the callus was obtained but it could be synthesized by the cells in static cultures⁴⁾ as well as suspension cultures. It is thus observed that the cells while growing in liquid medium still retain the potentialities to biosynthesize diosgenin and solasodine. The yield of these metabolites is, however, more in suspension cultures in *S. jasminoides* suggesting these conditions better for further study.

Acknowledgment. We are thankful to the Central Council for Research in Ayurveda and Siddha, New Delhi, India, for the funds.

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