STUDIES ON STEROIDAL SAPOGENINS FROM TISSUE CULTURES OF *AGAVE WIGHTII*

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ABSTRACT.—Eighteen-month-old unorganized callus tissue of *Agave wightii* Dr. & Prain, raised from seedlings on RT medium, was maintained as a static culture. This tissue, on transfer to liquid medium, produced bulbil-like growth even after the medium was supplemented with 2 ppm of 2,4-D. Both static and submerged cultures, when analyzed for their sapogenin content, yielded gitogenin, hecogenin, and tigogenin. The addition of cholesterol to the submerged culture medium resulted in a considerable increase in sapogenin content.

Steroidal sapogenins, an important group of compounds, have been reported to be present in tissue cultures of a number of plant species. Exhaustive reviews on this subject have appeared (1–3). Studies on the addition of precursors and/or hormones to the medium have shown a marked increase in the yield of these metabolites (4–6). *Agave wightii*, a good source of sapogenins, has not been studied *in vitro*. Because of these findings and because we wanted to continue our work on this plant species (7), the present work was undertaken. The addition of cholesterol to the liquid medium of suspension cultures of some other plant species resulted in marked increases in the yields of diosgenin, tigogenin, and gitogenin (4, 5). Since such studies have not been carried out for the production of hecogenin, we have studied the effect of different concentrations of cholesterol on the production of sapogenins, especially hecogenin, in a submerged culture of *A. wightii*.

MATERIAL AND METHODS

Unorganized callus tissue of *A. wightii* Dr. & Prain was raised from seedlings on revised (8) Murashige and Skoog's (9) medium (RT) supplemented with 1 ppm of 2,4-dichlorophenoxyacetic acid (2,4-D) and 1% agar. Tissue grown as static culture, when transferred to RT liquid medium containing 0.1 ppm of 2,4-D (60 ml of medium in a 150 ml flask on a rotary shaker at about 100 rpm) produced bulbil-like structures. Tissue was also grown on RT medium supplemented with 1 ppm and 2 ppm of 2,4-D, and this did not prevent organogenesis. Finally, liquid culture was maintained on RT medium containing 0.1 ppm of 2,4-D. The static and the submerged cultures were maintained for 18 months by frequent subculturings (every 6 to 8 weeks) in fresh RT medium. The tissue was harvested at the culture ages of 2, 4, 6, 8 and 10 weeks in the case of static culture and at 2, 4 and 6 weeks in the case of submerged culture. Different concentrations of cholesterol (30, 50 and 70 mg/100 ml of media) were added to submerged culture medium from the stock solutions of each of these concentrations in equivolumes (0.6 ml per flask) of 70% ethanol. The various tissue samples produced thereon were harvested at the ages of 2, 4 and 6 weeks and their growth indices (GI) were calculated (final dry weight of tissue-initial dry weight of tissue/initial dry weight of tissue) separately.

The tissues thus harvested were dried (at 100° for 15 min followed by 60° to constant weight) and powdered separately. Each powdered sample was hydrolyzed with 15% HCl in ethanol for 4 hr (10). The filtrate was extracted three times with ethyl acetate. The ethyl acetate fraction was washed with distilled water until neutral, brought to dryness, and reconstituted in chloroform for further analyses.

Each of the extracts so obtained was subjected to tlc (silica gel; benzene-ethyl acetate, 3:2) and then purified by preparative tlc in the same solvent system. Each corresponding band was eluted with chloroform and crystallized from acetone. The mp was obtained on each compound and each was subjected to ir spectral studies as given in an earlier publication (7).

The quantitative estimation of identified sapogenins was carried out colorimetrically with a spectrophotometer (Carl Zeiss, Jena DDR, VSU-2P) according to the method of Sanchez et al., (11). The results are the average of independent determinations of ten flasks (SE< 0.05%).

RESULTS

The callus tissue was compact and of creamish color but, on being transferred to liquid medium, the tissue showed a tendency to form bulbil-like structures (no rooting, fig. 1). The maximum growth index observed was 1.60 (table 1) in 6-week-old tissue in both instances. The growth index, however, decreased in

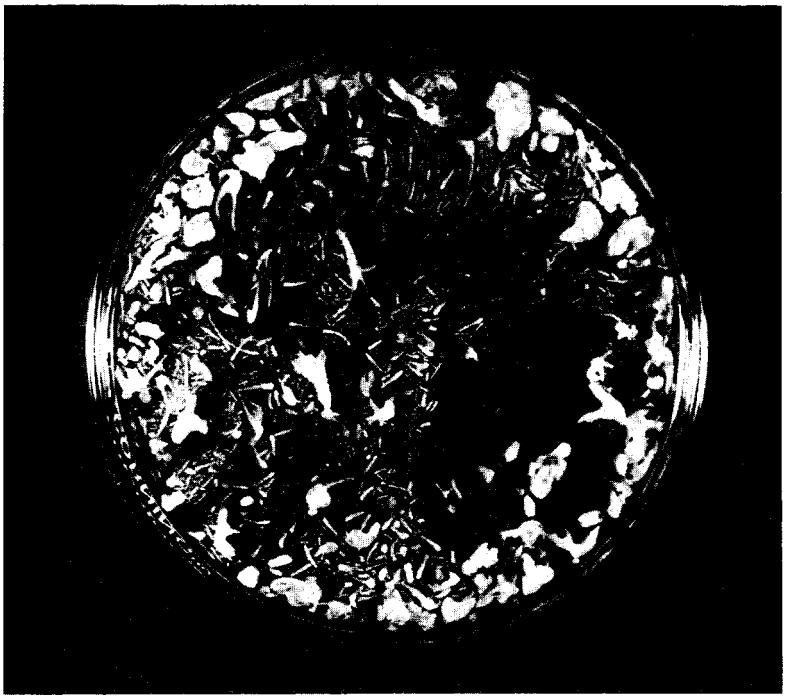

FIG. 1. Submerged culture of *A. wightii* showing bulbil-like structures.

cholesterol-fed submerged cultures with a maximum in the tissue fed with 30 mg/100 ml of medium (0.45, 0.67 and 0.95 in 2, 4 and 6 weeks respectively) and a minimum in the tissue fed with 70 mg/100 ml of medium (0.32, 0.25 and 0.66 in 2, 4 and 6 weeks respectively (table 2).

Sapogenins were identified as tigogenin, R_f 0.71, mp 206–208° (lit. (7) R_f 0.71, (12) mp 205–210°); hecogenin, R_f 0.58, mp 264–266° (lit. (7), R_f 0.58, (12) mp 268°) and gitogenin, R_f 0.28, mp 271–272° (lit. (7), R_f 0.28, (12) mp 271–272°) in each sample. The characteristic ir spectral peaks in all three sapogenins were found to be similar to those of their respective standard samples.

For tissues grown in static culture, the maximum total yield (on dry weight basis) of sapogenins (0.70%) as well as that of individual sapogenins (tigogenin 0.43%, hecogenin 0.16% and gitogenin 0.11%) was observed after 6 weeks of incubation. For tissue grown in submerged culture, the maximum total yield of sapogenins (0.62%) as well as that of the individual sapogenins (tigogenin 0.36%, hecogenin 0.13% and gitogenin 0.13%) was observed after 4 weeks of incubation (table 1).

Cholesterol-fed submerged cultures showed a marked increase in total sapogenin content (2.00%) in 4-week-old tissue fed with 70 mg/100 ml of medium. How-

TABLE 1. Sapogenin contents (% on dry weight basis) of *A. wightii;* static and submerged cultures.

Age of culture (weeks)	Static Culture (RT Medium)					Submerged Culture				
	GI[a]	Tigogenin	Hecogenin	Gitogenin	Total Sapogenin	GI[a]	Tigogenin	Hecogenin	Gitogenin	Total Sapogenin
2	0.30	0.26	0.05	0.02	0.33	0.50	0.27	0.07	0.04	0.40
4	0.67	0.32	0.09	0.04	0.45	0.90	0.36	0.13	0.13	0.62
6	1.60	0.43	0.16	0.11	0.70	1.60	0.28	0.12	0.08	0.48
8	1.10	0.40	0.14	0.13	0.67	—	—	—	—	—
10	1.00	0.21	0.09	0.07	0.38	—	—	—	—	—

[a]=GI=Growth Index (final dry weight of tissue—initial dry weight of tissue/initial dry weigh of tissue).
Each value represents an average of ten flasks.

ever, the high yield of individual sapogenins varied (table 2). While tigogenin was maximum (1.89%) in tissue fed with 70 mg of cholesterol/100 ml of medium, hecogenin and gitogenin showed maximum yields (0.15% and 0.13% respectively) in tissue fed with 50 mg of cholesterol/100 ml of medium (table 2).

TABLE 2. Effect of cholesterol feeding on production of sapogenins (% on dry weight basis) in *A. wightii* submerged culture.

Age of culture (weeks)	Conc. of cholesterol mg/100 ml of medium	RT Medium+Cholesterol				Total Sapogenin content
		GI[a]	Tigogenin content	Hecogenin content	Gigogenin content	
2	30	0.45	0.41	0.07	0.07	0.56
	50	0.33	0.22	0.06	0.04	0.33
	70	0.32	0.33	0.10	0.11	0.54
4	30	0.67	1.11	0.08	0.12	1.32
	50	0.45	1.50	0.15	0.13	1.78
	70	0.25	1.89	0.09	0.02	2.00
6	30	0.95	0.44	0.13	0.04	0.61
	50	0.80	0.67	0.15	0.13	0.95
	70	0.66	0.37	0.12	0.05	0.55

[a]=GI=Growth Index (final dry weight of tissue—initial dry weight of tissue/initial dry weight of tissue).
Each value represents average of ten flasks.

DISCUSSION

Khanna *et al.* (7) have reported tigogenin, hecogenin, and gitogenin (0.44%, 0.10% and traces respectively) from leaves of *A. wightii.* In the present study, callus tissue (static culture) showed a higher content of total as well as individual sapogenins (except tigogenin 0.43%) indicating that the tissue culture system is able to biosynthesize these substances in higher amounts. However, the contents in submerged culture showed lower concentrations, possibly due to the differentiation.

An increase in the yield of the total sapogenin content (2.00%) in cholesterol-fed tissue when compared with that of the control (0.70%) suggests that differ-

entiated tissue of *A. wightii* is capable of absorbing cholesterol when it is supplemented in the RT medium. But it is noteworthy that the amount of hecogenin also increases in the tissue when grown on RT medium supplemented with cholesterol.

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