DISTRIBUTION AND VARIATION OF DIOSGENIN IN DIFFERENT PARTS OF *COSTUS SPECIOSUS*

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Diosgenin, a steroidal sapogenin, is one of the most important raw materials as a precursor for the synthesis of a number of steroidal drugs which include corticosteroids, sex hormones, oral contraceptives, and anabolic agents.

Most of the present world supply of diosgenin is obtained from the rhizomes of *Dioscorea* spp. Because of a considerable increase in demand of steroidal drugs, a large number of plants have been screened to find alternative sources of diosgenin. Dasgupta and Pandey (1) were the first to report that rhizomes of *Costus speciosus* (Koen.) Sm., a perennial herb distributed throughout India, contained 2.12% diosgenin in 3.86% of total sapogenins. They also reported the presence of free diosgenin and tigogenin in its rhizome. Further, Rathore and Khanna (2) reported that rhizomes of *C. speciosus* also contained lanosterol and stigmasterol besides the diosgenin found in the stem (3). Free diosgenin, however, has not been reported in any other part of the plant except the rhizome. Similarly, the combined diosgenin has not been reported in the leaves and flowers. The distribution of both free and combined diosgenin in the different parts of the plant throughout the growing season has not been studied.

In view of the high diosgenin content in *C. speciosus* attempts have been made to cultivate the plant on a large scale. In order to develop suitable agropractices, it is important to know the optimum time of harvesting and dynamics of diosgenin accumulation. With a view to finding out the course of diosgenin synthesis and accumulation, we undertook an investigation of the distribution of diosgenin at different stages of its growth and in every part of the plant during its life cycle.

In the present communication, the distribution of diosgenin, both free and combined, has been studied in rhizomes, stems, leaves, flowers and seeds of the plant. Since the saponins are found in varying amounts in all parts of the plant, their relative abundance has been studied at regular intervals to determine the optimum time of accumulation and, hence, the optimum yield of diosgenin per unit area.

EXPERIMENTAL

Plant material.—*Costus speciosus* (Koen.) Sm. raised in our experimental farm from a single clone obtained from Rae Bareli district in U.P., India, during the month of March was used in the present investigation after its botanical identification by our Botany Division. Herbarium samples are on file in the herbarium of this institute.

For each experiment, 3 plants were picked at random. Different parts of the plant such as rhizomes, stems, leaves, flowers, and seeds were separated and cut into small pieces; the corresponding parts from the different plants were mixed. Three samples of each lot were combined, dried, powdered, and analyzed, and the average results recorded. The yield of diosgenin was calculated, unless specified to the contrary, on a dry weight basis.

Extraction of diosgenin.—A method suggested by Morris *et al.* (4) with modifications (5) was employed in the extraction of diosgenin from the various parts of the plant. Each of the weighed samples was extracted with *n*-hexane (8 hr) in a Soxhlet extractor for free diosgenin. It was then hydrolyzed by refluxing (4 hr) with 2.5 N HCl (1 g/10 ml). The hydrolyzed material was filtered and the residue on the filter

paper was washed with water till free from acid and soluble impurities. The residue was then oven dried at 80–100°, powdered, and extracted with *n*-hexane for glycosidic diosgenin.

THIN LAYER CHROMATOGRAPHY.—Pure diosgenin (3.4 mg) was dissolved in 100 ml of chloroform from which 1 ml, 2 ml, 3 ml, 4 ml and 5 ml representing (34 x 1) μg, (34 x 2) μg, (34 x 3) μg, (34 x 4) μg and (34 x 5) μg of diosgenin, respectively, were concentrated and subjected to tlc on a previously activated glass plate coated with silica gel-G (BDH) and developed with benzene-acetone (9:1). Diosgenin spots were visualized by spraying with Liebermann-Burchard reagent. A curve was plotted between the amount in the spot and the mass of the corresponding cut out strips. A straight line (fig. 1) supports the view that the larger

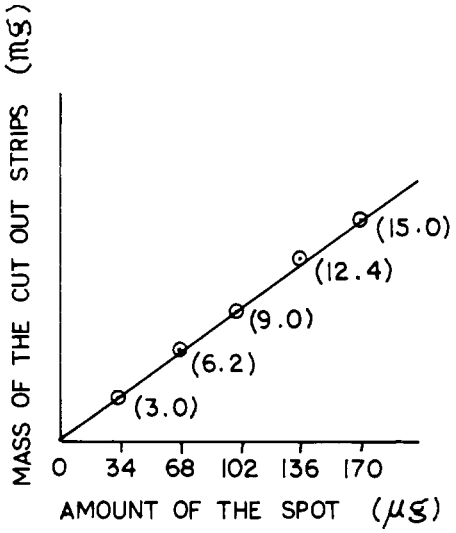

FIG. 1. Standard curve between the mass of the cut out strips in proportion to the concentration of charged diosgenin.

the amount the larger the spot. The extracts obtained by both the aforesaid ways were spotted on tlc plates, developed in the solvent system as previously described, and visualized by spraying with Liebermann-Burchard reagent. The visualized spots, including that of diosgenin, were traced on a superimposed transparent paper of uniform thickness. The areas corresponding to the spots were cut out and weighed together to obtain the total steroid content. The spot pertaining to diosgenin was also separately weighed. The accuracy by the paper weight measurement method was found ± 5% on total diosgenin content. A known amount of crude and of authentic diosgenin were applied separately on a plate, and preparative tlc was carried out in benzene-acetone (9:1). The portion of the chromatogram containing crude diosgenin was covered, and the rest of the portion containing authentic diosgenin was sprayed with LB reagent. The spot corresponding to diosgenin was cut and eluted with chloroform. The solvent was completely removed and the diosgenin weighed. The recovery of diosgenin by the elution procedure was about 82%. The results obtained by these two procedures are given in table 1.

RESULTS AND DISCUSSION

During the course of diosgenin extraction, β-sitosterol and some other steroids were also detected but are not listed in table 2 as they were in trace amounts. Diosgenin was differentiated and determined from the aforesaid steroids, both by preparative tlc and by relative paper weight measurement methods, as described in the experimental section. The results summarized in table 1 reveal

TABLE 1. Comparison of diosgenin content by two methods.

Sample No.	Diosgenin %	
	By preparative tlc	By paper wt. measurement
Rhizome (1)	0.07	0.08
Rhizome (2)	0.07	0.08
Seed (1)	0.18	0.19
Seed (2)	0.09	0.09
Flower (1)	0.01	0.01
Flower (2)	0.19	0.19
Stem (1)	0.18	0.19
Stem (2)	0.14	0.13
Leaf (1)	0.05	0.04
Leaf (2)	0.06	0.06

no appreciable variation in diosgenin content when these two methods were used. Since the preparative tlc method was more lengthy, the relative paper weight measurement method was employed in subsequent studies.

The results of the analysis of 27 samples consisting of various parts of the plants collected at different intervals are presented in table 2. Flower buds appeared in the first week of August, and they were in

TABLE 2. Diosgenin content in various parts of *C. speciosus* at different intervals from the initiation of flowering to dormant stage.

Date of Collection	Samples	Moisture %	Diosgenin %		
			glycosidic	free	total
18.8.78.....	Rhizome	95.0	2.55	0.08	2.63
	Stem	95.0	0.22	0.19	0.41
	Leaf	94.0	0.11	0.04	0.15
	Flower	96.0	1.04	0.01	1.05
	Seed	95.0	1.60	0.19	1.79
5.9.78.....	Rhizome	93.0	2.40	0.08	2.48
	Stem	94.0	0.31	0.13	0.44
	Leaf	84.6	0.15	0.06	0.21
	Flower	40.0	1.02	0.19	1.21
	Seed	70.0	1.71	0.09	1.80
25.9.78.....	Rhizome	91.5	1.51	0.08	1.59
	Stem	93.0	0.52	0.13	0.65
	Leaf	84.0	0.27	0.05	0.32
	Seed	40.0	2.36	0.45	2.81
13.10.78.....	Rhizome	86.4	1.26	0.05	1.31
	Stem	91.4	0.36	0.16	0.52
	Leaf	64.0	0.28	0.09	0.37
	Seed	32.5	2.24	0.31	2.55
30.10.78.....	Rhizome	85.6	1.14	0.04	1.18
	Stem	87.0	0.32	0.16	0.48
	Leaf	56.0	0.30	0.07	0.37
	Seed	27.5	1.75	0.37	2.12
16.11.78.....	Rhizome	85.3	1.04	0.05	1.09
	Stem	70.0	0.32	0.12	0.44
	Seed	13.3	1.33	0.19	1.52
3.12.78.....	Rhizome	85.2	1.04	0.15	1.19
	Stem	60.0	0.26	0.17	0.43

full bloom by mid August. The flowers dropped after a fortnight and the seeds borne in capsules matured towards the end of November; the aerial parts of the plant died out in December leaving the plant in the dormant stage.

Certain facts are revealed from the pattern of distribution of diosgenin as depicted in table 2. There is a constant drop of diosgenin content in the rhizomes from early flowering stage (maximum) to the minimum, when the seeds are mature. The diosgenin is distributed unevenly in the leaves and stems. The diosgenin in these parts varies only to a small extent during its growth, whereas an appreciable variation is observed in the diosgenin content of the seeds.

Presumably, the flowers, being involved in the reproduction process, accumulate a high quantity of diosgenin, which is ultimately transferred to the seeds, resulting in a relatively high percentage of diosgenin content. It increases with the development of the seeds, but when they attain full maturity there is a gradual decrease in the diosgenin content.

The relatively high percentage of free diosgenin in the stems and leaves and the low percentage in the rhizomes support the view that glycosidation is probably a later process in the biosynthesis of the saponin formation. Saponins appear to be stored by the plant during its dormancy both in the rhizomes and seeds.

In view of the above, the conclusion is drawn that diosgenin accumulates in seeds and rhizomes, whereas other

parts of the plant act for translocation of diosgenin. The crop should be harvested during the dormant period of the plant as the biomass of its rhizome is maximum (6) at this stage, resulting in a higher yield of diosgenin per unit area. Seeds should be collected when they are dry. No other parts of the plant can be commercially utilized as they contain only trace amounts of diosgenin. However, the rhizome at the flowering stage of the plant can not be exploited, although it contains a maximum percentage of diosgenin at this stage, because of a considerable decrease in their biomass (6) that would give, on an average, a low yield of diosgenin.

The yield of rhizomes (21 months) has been found to be 8.25 tonnes (d.w.b), whereas the yield of seeds is 1 tonne (d.w.b) per hectare (7). Thus, the farmers can get 98.18 kg @ 1.19% and 15.20 kg @ 1.52% of diosgenin per hectare from rhizomes and seeds, respectively, by a 21 months crop.

ACKNOWLEDGMENT

The authors are grateful to Dr. Akhtar Husain, Director, CIMAP, Lucknow, for his valuable suggestions and keen interest throughout the investigation.

Received 6 March 1980

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