COSTUS SPECIOSUS RHIZOME AS SOURCE OF DIOSGENIN

Y. K. SARIN, K. L. BEDI AND C. K. ATAL

Regional Research Laboratory, Jammu-Tawi

ABSTRACT

Rhizomes of *Costus speciosus* Sm., a perennial herb having a wide distribution in India, have been found to contain upto 2·6% of diosgenin. The plant can be easily propagated through rhizome cuttings and have good prospects of becoming a raw material for steroid industry in India.

INTRODUCTION

DIOSGENIN is one of the sapogenins extensively used in the manufacture of steroidal hormones. The raw material for it consists mainly of the underground parts of a few species of *Dioscorea*, occurring wild in Mexico, South America, India and China. The demand for steroidal hormones, especially those used as antifertility drugs has increased in recent years and the fears are expressed about an early exhaustion of the raw materials due to difficulties in cultivating high yielding varieties of *Dioscorea*. A search for substitute raw materials is being carried out in different parts of the world but none of the new plants found to contain diosgenin have provided enough evidence of becoming a commercial success. In India, rhizomes of *Dioscorea deltoidea* Wall. and *Dioscorea prazeri* Prain and Burkill. occurring wild in Himalayas (diosgenin content of 3·5% and 2·0% respectively)[3] are the only raw materials utilized by steroid industry. The supplies available from these two sources are however likely to exhaust in the next ten to fifteen years due to large collections from the forest areas, poor natural regeneration and failure in raising these plants as commercial crops. A number of other plants, *viz.*, *Trillium govanianum, Balanites roxburghii* and *Parispolyphylla* have been reported to contain diosgenin[2,4,5] but there is hardly any scope for their commercial exploitation due to a very low diosgenin content or poor availability. Recently Das Gupta and Pandey[1] reported the occurrence of diosgenin in the rhizomes of *Costus speciosus* Sm., a perennial herb commonly available in different parts of India. This report led the authors to undertake detailed investigations on utilization of the plant for commercial production of diosgenin.

BOTANICAL CHARACTERISTICS

Costus speciosus Sm. (Fam. : Zingiberaceae) is an erect herbaceous plant upto 2 m high with long lanceolate leaves and white fragrant flowers in terminal clusters. The plant flowers during the months of July and August, the aerial parts withering away during winter months. It has a very wide distribution in India, occurring throughout the sub-Himalayan tract from Himachal Pradesh to Assam, Vindhya and Satpura hills in Central India and the western ghats of Maharashtra, Karnataka and Kerala. It is more commonly found in the moist shady localities under the mixed deciduous forests upto an elevation of 1200 m above mean sea level.

DIOSGENIN CONTENT OF THE RHIZOME

Detailed chemical examinations of the rhizome was done for this purpose. In the first experiment 250 g of dried rhizomes were hydrolysed with 10% hydrochloric acid, filtered, washed and exhausted with *n*-hexane. The extract on concentration gave 5 g (2%) of diosgenin needles (201° C–203° C). These when crystallised from ethyl alcohol yielded fine needles (205° C–206° C) showing no depression in melting point when mixed with authentic samples of diosgenin. Further the benzene and chloroform extracts yielded nothing whereas the alcoholic extract designated as fraction A showed one major spot moving with the solvent front on T L C (Silica gel ; hexane : ethyl acetate :: 80 : 20). This fraction was further subjected to resolution through different solvent systems (benzene R_f 0·25 ; *n*-hexane : ethyl acetate 95 : 5, R_f 0·47 ; petroleum ether : 90 : 10 R_f 0·53), but it always showed the major spot to be a single entity. In another experiment, 300 g of dried tubers were defatted with petroleum ether and then exhausted with 85% alcohol. The alcoholic extract was concentrated to remove as much alcohol as possible and the residue hydrolysed and processed as usual. This yielded about 7·5 g (2·5%) of a brownish solid designated as fraction B which (*n*-hexane : ethyl acetate ; 80–20): revealed four spots on T L C, one corresponding to authentic diosgenin (R_f 0·51), another moving with the front (R_f 0·95) and the rest two minor spots with R_f values as 0·56 and 0·87. It was then chromatographed over alumina (110 g) when *n*-hexane fraction gave a mixture of the R_f 0·87 and 0·95 spots, the latter very much predominating (fraction C). The spot with R_f 0·95 when run concurrently with fraction A was found to be identical with major spot in the latter and moved parallel in all the solvent systems studied above. Subsequent elution of the column with 10% benzene in petroleum ether gave predominantly diosgenin (1·95%) admixed with R_f 0·56 spot (fraction D). This last fraction on single crystallization from ethyl alcohol gave white needles

(m.p. 204° C–205° C) which when mixed with authentic diosgenin showed no depression. As fractions A and C were similar in T L C pattern, they were combined and rechromatographed over alumina. The *n*-hexane fractions yielded a white crystalline material which on a single crystallization from ethyl alcohol gave white needles positive to Liebermann Burchard test for sterols.

Das Gupta and Pandey[1] obtained 3·88% of an extractive (ethyl alcohol) which they designated as sapogenins. This further yielded 2·12% of pure diosgenin. In the present investigation the percentage of pure diosgenin (m.p. 204° C–205° C) obtained is about 2% out of a total extractive of 2·5%. The above workers did not investigate the nondiosgenin part which could have been sapogenins or a mixture of sapogenins and some other compounds because the rhizomes were extracted with ethyl alcohol. Further elucidation work on this fraction is in progress. It was further observed that the quality of diosgenin obtained from *Costus speciosus* is better than that obtained from *Dioscorea deltoidea* (whiter colour and a melting point of 201° C–203° C against 190° C of diosgenin obtained from the latter). Comparative T L C of the diosgenin obtained from the two plants confirmed the above observations.

Raw Material Resources

Reconnaissance carried out in various parts of India revealed a fair availability of *Costus* rhizomes in the sub-Himalayan tract of Himachal Pradesh, Uttar Pradesh and Nepal in the north and the Ghat areas of Maharashtra, Goa, Karnataka and Kerala in western and southern India. A lot of variation in diosgenin content ranging from 0·38% to 2·6% has been noticed in rhizome samples collected from various localities. The samples obtained from the sub-Himalayan tract have higher diosgenin content than those from western and southern India. A further investigation on the diosgenin variability was carried out. Rhizome samples from 20 plants growing side by side were collected from 2 different localities (Kangra in Himachal Pradesh and Dehra Dun in Uttar Pradesh) and chemically analysed. The diosgenin content was found to vary from plant to plant ranging from 0·58% to 2·63%.

Possibilities for cultivating high yielding strains (diosgenin content 2% or more) were therefore examined. Rhizomes of the plant were cut in small pieces (each weighing about 35 g) and were put in well-prepared soil in rows at a distance of 0·5 m from plant to plant as well as from row to row during the month of March. These pieces sprouted in the last week of May. The plants attained a height of about 1 m towards the end of July when profuse flowering also ensued. The growth of plant was comparatively better in well-irrigated plots. The aerial portions of the plant withered away in December and rhizomes remained dormant till next May. To check the increase in weight in the underground parts the rhizomes were dug up in the month of January, *i.e.*, after 9 months of planting. These had attained a good size and weighed 1570 g on an average. The increase in size from 35 g (seed tuber) to 1570 g (maximum harvest) was very encouraging. The rhizomes on chemical analysis, however, showed a decline in diosgenin content from 2·3% to (average) 1·6%. Experiments are in progress to determine the correlation between the age of the plant and the diosgenin content.

Conclusions

The present investigations reveal great potentialities for utilization of *Costus speciosus* rhizomes as a substitute raw material for the production of diosgenin in India. The yield of diosgenin is comparatively low, being 1·5% to 2% against 3% to 4% in the rhizomes of *Dioscorea deltoidea* and 2% in the rhizomes of *Dioscorea prazeri*. There are, however, a number of points which well compensate the low yield of diosgenin. These are

1. The plant has a very wide distribution zone extending from sub-Himalayan tract in the north to hilly regions of Central India and the western ghats, and can be collected in appreciably large quantities.
2. The diosgenin obtained from it is far more pure than that obtained from *Dioscorea* spp.
3. The plant can be easily propagated even in the plains. Preliminary cultural trials show a very fast growth of underground portions with a slight decline in diosgenin content.

As stated above, the supplies of *Dioscorea* rhizomes are fast depleting due to large scale extraction from the forest areas. The situation is further aggravated due to failures in raising Indian *Dioscoreas* as commercial crops. In the light of these facts the development of *Costus speciosus* Sm. as a raw material for steroid industry becomes more important. Attempts towards large scale introduction of clones with a diosgenin content of 2% or above are being made together with technoeconomic studies for commercial utilization of this new raw material. The findings will form the subject-matter of a further communication. The authors thank Shri Vir Singh and Shri J. P. Singh for assistance rendered in the work.

1. Das Gupta, B. and Pandey, V. B., *Experientia*, 1970, p. 26.
2. Dhekne, V. V. and Bhide, B. V., *Jour. Indian Chem. Soc.*, 1951, 28, 588.
3. Hardman, R., *Tropical Science*, 1969, 2 (3), 196.
4. Sarin, Y. K. and Kapoor, L. D., *Indian Jour. Pharm.*, 1965, 27 (1), 6.
5. Singh, A., Srivastava, S. N. and Kapoor, L. D., *Indian Jour. Chem.*, 1966, 4, 460.