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Economically Useful Plants of Sri Lanka

II* Commercially Important Steroidal Sapogenins from Sri Lanka Plants†

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Abstract : Several species of *Dioscorea*, *Costus*, *Agave*, *Yucca* and *Furcrea* growing in Sri Lanka have been assayed for saponins with a view to identifying suitable species and cultivars for commercially useful steroidal sapogenins. Rhizomes of *Costus speciosus* and the leaves of *Agave americana* have been identified as the best available materials in Sri Lanka for diosgenin and hecogenin, respectively.

1. Introduction

Sapogenins are hydrolysis products of saponins which are phytoconstituents widely distributed in the plant kingdom. Some sapogenins containing the steroid nucleus are useful starting materials in the commercial synthesis of physiologically and pharmacologically active steroids[1] such as corticosteroids, sex hormones and oral contraceptives. Commercially important steroidal sapogenins are present as saponins in some tubers of *Dioscorea*,[8] leaves of *Agave*,[8] and *Yucca*[8] species. Diosgenin (I) isolated from *Dioscorea* species is at present the principal starting material for the synthesis of the steroidal hormones *viz.*, testosterone, estradiol and progesterone.[5] The world requirement of diosgenin was estimated in 1973 to be around 1,000 tons per year.[1] Though this may be on the high side, there was in fact a world shortage in 1974.[1] Mexico was the world's leading producer of diosgenin and in 1968 it produced 500 tons.[5] India's contribution of diosgenin in 1968 was 30 tons. Chief sources of diosgenin in India were the tubers of *Dioscorea deltoidea* Wall and *Dioscorea prazeri* Prain & Burk.[5] The purchase price[5] of diosgenin in 1969 ranged from US $ 12/50 per kg to US $ 17 per kg. Whilst in 1976, Datta quoted[3] a price of Rs. (Indian) 1000/- per kg.

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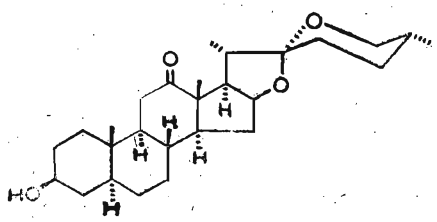
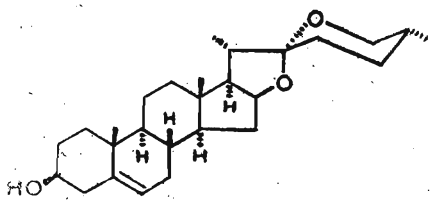
Hecogenin (II), a 12-keto steroidal sapogenin, though unsuitable for the manufacture of oral contraceptives is satisfactory for corticosteroid synthesis.[1] In fact 6% of steroid precursors used by the pharmaceutical industry is hecogenin.[1] The leaf of *Agave sisalana* Perrine, which is widely grown in Kenya and Tanzania for obtaining fibre, gives a juice from which hecogenin is extracted. The chief hecogenin producer in 1968 was Africa (40 tons).[6]

An alternative source for diosgenin (I) was discovered in 1970 by Das Gupta and Pandey[2] who showed that from the rhizomes of *Costus speciosus* (Koen.) Sm. growing in India about 2.1% of diosgenin could be obtained. In 1974, Sarin *et al.*[7] reported further studies on the rhizomes of *C. speciosus*. They found that the diosgenin content of these rhizomes varied from 0.58 to 2.63%.

Some *Dioscorea*, *Costus*, *Agave* and *Yucca* species are found growing wild and/or are cultivated in Sri Lanka. With the view to commercial exploitation for steroidal precursors, we have screened these and some related species *viz.*, *Furcrea foetida* and *F. watsonia*, for their saponin content.



(I) Diosgenin



(II) Hecogenin

2. Experimental

2.1. Froth test for Saponins

The plant part (100 mg) was chopped and was shaken with water in a test tube. If a persistent froth of 3 cm length existed for 1/2 h, then the test was considered to be positive.[4]

2.2. Extraction of Saponins

The plant part (0.5 kg) which showed positive froth test was chopped and was immediately extracted in a soxhlet with rectified spirit (1 l.) for 24 h. The residue obtained after evaporation of solvent was extracted in a soxhlet with light petroleum (40–60°) (250 ml) and the defatted residue was partitioned between *n*-butanol and water. The *n*-butanol layer was dried (Na_2SO_4) and evaporated. The weight percentage of the butanol soluble residue (saponin) was calculated. Haemolysis test[4] was performed on this residue as described below.

2.3. Haemolysis test[4]

A medium double layered plate of 5% bovine red cell suspension in isotonic phosphate buffer (pH 7.4) in 1.5% agar-buffer overlaid on 1.5% agar-buffer was used. A disc of filter paper saturated in the appropriate solution of the test substance dissolved in phosphate buffer and diluted serially two-fold in the same buffer was placed on the surface of the well dried plate. Another disc containing digitonin in place of the test substance was placed on the same plate. The plate was left for 24 h and haemolytic zone, if any, was observed.

2.4. Hydrolysis of Saponins for TLC assay

The saponin (100 mg) was treated with rectified spirit (10 ml) and 10 ml of 10% HCl. The mixture was heated under reflux for 4 h. Solvent was evaporated under reduced pressure and the residue was taken up in chloroform, washed with water and the organic layer was dried (Na_2SO_4). The organic layer after concentration was subjected to TLC analyses using chloroform or chloroform-methanol (9 : 1) as developing solvents and ceric sulphate spray as the locating reagent.

The above single hydrolysis was sufficient for the assay of diosgenin. However, for detection of hecogenin a double hydrolysis (see section 2.6. below) was employed.

2.5. Isolation of Diosgenin (I) from *Costus Speciosus*

The rhizomes (25 g) of *C. speciosus* were cut into small pieces and were heated (80°) with 50 ml of 10% HCl (w/w) for 4 h. After cooling, the hydrolysis product was extracted with chloroform (2 × 25 ml). The organic layer was dried (Na_2SO_4) and solvent evaporated. The residue was extracted with light petroleum (60°–80°) (10 ml). The light petroleum extract on concentration gave a solid (0.3 g) whose TLC analysis indicated diosgenin to be the major sapogenin. Preparative TLC of the above solid gave pure diosgenin (0.2 g, 0.8% from undried rhizomes) which was identified by m.p., mixed m.p. and co-TLC.

2.6. Isolation of Hecogenin (II)

2.6.1. *Hydrolysis of Agave americana leaf saponin*

Fresh *A. americana* leaves (1 kg) were cut into pieces and extracted in a soxhlet with rectified spirit (4 l) for 12 h. Solvent was evaporated under reduced pressure and the residue was refluxed for 2 h with ethanolic HCl (1M, 300 ml). The reaction mixture was filtered, solvent was evaporated and the residue was treated with 10% alcoholic KOH until solution was alkaline. The mixture was refluxed for 30 min, cooled and extracted with ether. The ethereal layer was dried (Na_2SO_4) and evaporated. The residue was crystallised from acetone to give pure hecogenin (1g, 0.1%), m.p. 258° – 260°. The hecogenin isolated was found to be identical with an authentic sample (co-TLC and mixed m.p.).

2.6.2. *Direct hydrolysis of Agave leaves*

Fresh *Agava americana* leaves (1 kg) were chopped and ground in a mortar. The slurry thus obtained was transferred into a flask and heated with 60% H_2SO_4 at 150° for 4 h. Hot filtration gave a precipitate which was further heated for 2 h with 20% H_2SO_4. The residue from hot filtration was washed with water, with 1% NaOH and again with water. It was dried and the residue was extracted with chloroform. The chloroform extracts on concentration gave hecogenin (II) (0.5g, 0.05%), m.p. 259°–261°, identical with an authentic sample (co-TLC and mixed m.p.).

3. Results and Discussion

3.1. Commercial source for Diosgenin (I)

3.1.1. *Screening of the Dioscorea species of Sri Lanka*

Eleven different samples of underground tubers or aerial bulbils of *Dioscoreas* distributed amongst eight different species were collected from different parts of the country (see Table 1). The saponins were extracted by the method given in Section 2.2. The weight percentages of the *n*-butanol soluble residues (saponins) are presented in Table 1. The *n*-butanol solubles were subjected to haemolysis test only if the yield was $\geqslant 1\%$, since the underground tubers or aerial bulbils with a lower percentage will not be of economic importance. Those residues showing positive haemolysis test were subjected to hydrolysis. The hydrolysates were compared on TLC with authentic samples of diosgenin (I) and hecogenin (II). The results are given in Table 1. Out of the *Dioscorea* species assayed, only *angili ala* can be considered as a source for the commercially useful diosgenin. However, because of the low percentage of diosgenin, this *Dioscorea* species would not be of economic importance.

The two *Dioscorea* species, *D. deltoidea* and *D. prazeri* have been exploited in India. It is estimated that from these species growing at an altitude of 3000 to 8000 ft., 90 kg of diosgenin per acre per year could be obtained.[3] Two other species growing in the plains, *D. composita* Hemsl. and *D. floribunda* Mart. & Gal. have also been exploited[3] in India for diosgenin. According to trials carried out in India, the net profit from one acre of plantation per year, after extraction of diosgenin has been estimated to be around US $ 7,000/-. However, there is no record of these high diosgenin yielding *Dioscorea* species in Sri Lanka. Our results indicate that since the local *Dioscoreas* assayed for diosgenin, which included some wild types, are not of economic importance, attempts should be made to introduce the Indian varieties, at least as intercrops in tea, rubber or coconut plantations. Since the saponin content could vary with locality and climatic conditions (see Table 2), prior to large scale cultivation, it may be necessary to estimate the saponin content of the Indian species once introduced to Sri Lanka.

TABLE 1. % Saponins of some *Dioscorea* species growing in Sri Lanka

Species	Place of Collection	Plant part	Yield of Saponin (%)	Haemolysis Test†	Sapogenin
D. alata L (Jaffna purple)	Gannoruwa	Tubers	1.0	—	
D. alata L (*Sin.* Kahata ala)	Gannoruwa	Tubers	0.38		
D. alata L (*Sin.* Kahata ala)	Atabage	Tubers	1.0	—	
D. bulbifera L (*Sin.* Udala)	Atabage	Aerial bulbils	0.75		
D. bulbifera L (*Sin.* Udala)	Atabage	Tubers	2.2	+	No diosgenin, No hecogenin
D. bulbifera L	Jaffna	Tubers	1.65	±	No diosgenin, No hecogenin
D. esculenta (Lour) Burk. (*Sin.* Kukulala)	Gannoruwa	Tubers	1.0		
D. esculenta (Lour) Burk. (*Sin.* Kiriala)	Atabage	Tubers	0.20		
D. pentaphylla (*Sin.* Katuala)	Atabage	Tubers	0.88		
Dioscores sp.* (*Sin.* Angiliala)	Atabage	Tubers and aerial bulbils	1.0	++	Very small amount of diosgenin.
Dioscorea sp.* (*Sin.* Java ala)	Atabage	Tubers	0.32		

*The botanical names of these species are not available.

†No haemolysis, — ; weak haemolysis, + ; strong haemolysis, ++.

3.1.2. *Screening of the Costus species of Sri Lanka*

Two different species of *Costus*, *viz. C. speciosus* and *C. afer* have been identified as growing in Sri Lanka. Several specimens of these were collected from different parts of the country. Saponins from the rhizomes were isolated using the method given in Section *2.2*. The haemolysis test did not yield satisfactory results. Since the saponin content of most of the rhizomes studied were $> 1\%$ the extracts were subjected to hydrolysis. The sapogenins were first assayed by TLC and the results indicated that all saponins of the *Costus* rhizomes studied had diosgenin as the major steroidal sapogenin (see Table 2). The presence of diosgenin in the hydrolysate was further confirmed by isolation. Diosgenin was also isolated in good yield from the *Costus* rhizomes by a direct hydrolysis route (see *2.5*). The *Costus* species, especially *C. speciosus*, which grows wild in Sri Lanka is a good source of diosgenin in Sri Lanka. According to the economics of its cultivation in India[3], one acre of land can give about 4000 kg of the dried rhizomes of *C. speciosus* per year. This on processing can yield 15 to 30 kg of diosgenin even if the yield of saponin is only 1%. According to the 1976 purchase price[3] this would fetch US $ 1875 to $ 3750. Considering this to be an annual turnover for an acre plot cultivated, the foreign exchange earning would be in the region of Rs. (Sri Lanka) 28,725 to Rs. 57,450 per year. The cost of cultivation and extraction of diosgenin has not been considered in this computation. Considering the cheap labour available and the possibility of extracting diosgenin from the rhizomes by a direct hydrolysis procedure which could be employed (see *2.5*), the total cost of labour and cost of extracting diosgenin in Sri Lanka cannot be much more than the Rs. (Indian) 12,000 estimated in India[3] for a 1.0% yield of saponin from the rhizomes. As an initial experiment, we suggest that *Costus speciosus* should be cultivated as an intercrop in rubber estates.

TABLE 2. % Saponins from the *Costus* species growing in Sri Lanka

Species	Place of Collection	Yield of Saponin (%)	Haemolysis Test	Froth Test
C. speciosus (Koen.) Sm.	Gilimale	1.4	+*	+
C. afer	Kandy	0.3	—	+
C. speciosus	Kandy	0.7	—	+
C. speciosus	Deraniyagala	2.5	—	+

*Weakly positive.

Note : Diosgenin (I) has been shown to be present in the hydrolysates of all the saponin extracts by TLC and from the sample of *C. speciosus* from Deraniyagala, (I) has been isolated and identified. In all the cases, rhizomes contained saponins.

3.2. Commercial source of hecogenin (II)

Phytochemical screening of the *Agave*, *Yucca* and *Furcrea* species growing in Sri Lanka was undertaken with the view to identifying the best source of hecogenin. The species studied are listed in Table 3. *Agave americana* was found to be the best source of hecogenin. The fresh leaves contain *ca.* 1.5% of saponin. Hydrolysis of this gave hecogenin in a reasonable yield. The conventional method involving extraction of saponin using solvents is too expensive and is not economically viable. The economically feasible route would be to chop the leaves and heat with 60% H_2SO_4 and effect the hydrolysis before extracting hecogenin (see *2.6.2.*).

TABLE 3. Results of the screening of *Agave*, *Yucca*, and *Furcrea* species of Sri Lanka for their saponin contents.

Species*	Plant part†	Yield of Saponin (%)	Haemolysis test‡	TLC of sapogenin mixture	
				No. of spots	Hecogenin
Agave americana L Var. Variegata	leaves	1.5	++	4	+††
A. angustifolia Haw.	fruits	1.1	++	6	+
A. franzosoni Baker	fruits	0.6**			
A. rigida Mill Var, Sisalana Engl.	leaves	1.1	—		
A. toquilana Wood	leaves	0.3**			
Yucca aloifolia L	leaves	2.3	+	3	+
Y. gloriosa L	leaves	7.2	+	3	+
Furcrea foetida (L) Haw. (= *F. gigantia* Vent.)	leaves	1.0	++	4	+
F. watsonia Hort.	fruits	0.5**			

* All the plant parts were collected in and around Peradeniya.

† Fresh plant parts were used in the extraction.

‡ No haemolysis, — ; weak haemolysis, + ; very strong haemolysis, ++.

** No further analysis was performed as the saponin yield was low (< 1%)

†† Isolated and identified by comparison with an authentic sample.

4. Conclusion

The results indicate that none of the locally growing *Dioscorea* species including some wild ones studied is suitable as a commercial source of diosgenin. If diosgenin rich Indian *Dioscorea* species, *viz. D. deltoidea, D. prazeri, D. composita* and *D. floribunda* prove to be good sources of diosgenin under local conditions, steps should be taken to introduce them for commercial exploitation.

Costus species growing wild in Sri Lanka which are shown to be rich in diosgenin should be cultivated on an organised scale preferably in the rubber growing districts.

Agave americana is a good source of hecogenin in Sri Lanka. Cultivation of this should be encouraged in semi-arid and agriculturally unproductive areas.

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