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SCREENING OF PHILIPPINE PLANTS FOR STEROIDAL SAPOGENINS, I

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ONE TEXT FIGURE

INTRODUCTION

Cortisone, scientifically known as 17-hydroxy-11-dehydrocorticosterone, is an adrenal cortical hormone. It was originally isolated from the cortex of adrenal glands and later synthesized from bile acids of cattle. After its isolation by Kendall, Dr. Hench(6) of the Mayo Clinic headed the group that pioneered its clinical use. Chemists of Merck and Co. participated in the biochemical investigations that resulted in its partial synthesis.

Stimulated by the encouraging effects of cortisone on rheumatic diseases, the search for cheap, abundant, suitable starting materials for its synthesis was intensified. As this hormone belongs to a group of compounds known as steroids, the importance of some saponaceous plants as a possible source of materials for its synthesis is recognized. Steroidal sapogenins occur in plants in a combined glycosidal form which can be cleaved by the use of strong hydrochloric acid. Consequently, the completeness of the acid hydrolysis of the precursor saponins determines the yield of sapogenins.

Marker and Applezweig(4) found that a number of plants growing in Mexico contain steroidal sapogenins and, therefore, provide a source for the synthesis of cortical steroid. Plant families investigated by Marker, et al. include Amaryllidaceæ,

Dioscoreaceæ, Apocynaceæ, and Liliaceæ. Since most of the above-mentioned plant families are also represented in the Philippines, the screening of local plants was undertaken.

This preliminary report includes some species of the families Leguminosæ, Dioscoreaceæ, Amaryllidaceæ, Apocynaceæ, Euphorbiaceæ, Liliaceæ, and Malvaceæ. Plants belonging to other families are scheduled for later screening.

In the course of screening hundreds of plant samples for steroidal sapogenins by Wall, et al.,(8) it became imperative to find a procedure which could be used on large numbers of samples. A much more rapid micro-screening procedure was used since macro-isolation is time-consuming and many species were negative.

EXPERIMENTAL PROCEDURE

Plant samples used in the investigation were collected from the different places in the Philippines and identified according to families, genera, and species. The collection of such plant samples was primarily based on the plants listed by Quisumbing(5) and Brown.(1)

The method finally adopted was based on the studies of Diaz, et al.(2)

Extraction.—The plant material was dried in the oven and powdered in a mortar. An aliquot sample of at least 10 grams was covered with 60 ml of 80 per cent ethyl alcohol and refluxed for 1 hour. The sample was then cooled, filtered, washed, and made to a final volume of 100 ml with 80 per cent ethyl alcohol.

Hemolytic detection of saponins.—Blood Standardization. Ten to 20 ml of whole cow's blood was suspended in 100 ml of 0.85 per cent aqueous sodium chloride solution, the suspension was centrifuged, and the supernatant liquid was decanted. The process was repeated twice. The blood corpuscles were then suspended in 400 ml of 0.85 per cent sodium chloride solution. Ten millimeters of the turbid suspension and 1 ml of digitonin solution (10 mg of pure digitonin in 100 ml of 80 per cent ethyl alcohol) were mixed in a 15-ml conical centrifuge tube. The mixture was kept at room temperature for 5 minutes, and then visually compared with a tube of untreated blood suspension. If complete hemolysis had occurred, the tube containing digitonin would be entirely clear.

In our experience, complete hemolysis by 1 ml of digitonin did not occur. Hence the stock blood suspension was progressively

diluted with 0.85 per cent sodium chloride solution, until 10 ml of the blood suspension were completely hemolyzed at room temperature within 5 minutes.

Detection of saponins.—One milliliter of the plant extract was added to 10 ml of standardized blood suspension. After 5 minutes, the presence or absence of hemolysis was observed. Samples giving a negative test were discarded. Positive extracts were used for detection of steroidal sapogenins.

Isolation of crude sapogenins.—An aliquot of the alcoholic extract equivalent to 5.0 grams or original plant material (moisture-free basis) was concentrated and defatted with benzene saturated with 50 per cent ethyl alcohol. The sapogenins were hydrolyzed with 4 N hydrochloric acid at 75 to 80°C for two hours and the resultant sapogenins extracted with benzene. The benzene extract containing crude sapogenins was placed in a small beaker and evaporated on a steam bath, 2 ml of acetic anhydride were added and the mixture was gently boiled for several minutes. After acetylation, 5 ml of benzene and 5 ml of methanol saturated with potassium hydroxide were added and the contents were mixed vigorously. Immediately, 5 ml of water were added and mixed well and the tube was centrifuged. The benzene layer which separated was withdrawn and the residual aqueous methanol was twice re-extracted with benzene. The combined benzene layers containing the crude sapogenin acetates were evaporated to dryness on a steam bath and dried to constant weight in a vacuum oven at 110°. A yield of crude acetate less than 10 mg meant less than 0.1 per cent pure sapogenin, and the sample was classified as negative for our purposes.

Absorption chromatography.—The sample was dissolved in 5 per cent chloroform in benzene, eluting with this solvent and then with 20 per cent chloroform in benzene in order to remove the monohydroxysapogenins from the column. Chloroform was then used to remove the dihydroxy sapogenins, followed by 20 per cent ethyl alcohol in benzene. Each eluent was collected in separate containers and the solvent was evaporated to dryness.

Spectrophotometric determination.—Henry A. Walens, et al.(7) showed that steroidal sapogenins, on treatment with concentrated sulfuric acid, give characteristic ultraviolet absorption spectra of the sulfuric acid chromogens in the region 220 to 400 mμ. This can be used in the detection and estimation of steroidal sapogenins.

TABLE 1. *Results of hemolysis test and estimated sapogenin content found in the plant collections.*

Species	Local name	Plant part[1]	Hemolysis test	Estimated total m.f.b.
LEGUMINOSÆ				*Per cent*
Abrus precatorius Linn.	Saga	*l*		0
Do	do	*s*		0
Do	do	*sd*	1	0
Acacia concinna (Willd.) DC.	Acacia	*l*	1	0.21
Acacia farnesiana (Linn.) Willd.	Aroma	*fr*	1	0.32
Do	do	*l*		0
Adenanthera intermedia Merr.	Tanglin	*sd*		0
Arachis hypogea Linn.	Mani	*sd*		0
Alysicarpus vaginalis	Mani-manian	*r*		0
Do	do	*l*	1	0
Bauhinia malabarica Roxb.	Alibangbang	*b*		0
Caesalpinia crista Linn.	Kalumbibit	*sd*	1	0.12
Caesalpinia sappan (Linn.)	Sapang	*b*		0
Caesalpinia pulcherrima (Linn.) Sw.	Caballero	*b*		0
Cassia alata Linn.	Acapulco	*l*		0
Cassia fistula Linn.	Fistula	*b*	1	0
Do	do	*s*	1	0.57
Do	do	*l*	1	0
Cajanus cajan (Linn.)Mill.	Kadios	*sd*	1	0
Do	do	*l*		0
Do	do	*s*		0
Cassia tora Linn.	Balatong-aso	*r*		0
Do	do	*s*		0
Do	do	*l*		0
Do	do	*fr*		0
Clitorea ternatea Linn.	Pukinggan	*l*		0
Do	do	*s*	1	0
Do	do	*rb*	1	0
Dalbergia cumingiana Benth.	Tahid-labuyo	*s*	1	0.36
Desmodium triflorum (Linn.) DC.	Kaliskis-dalag	*l*		0
Dolichos lablab Linn.	Batau	*fr*		0
Entada phaseoloides (Linn.) Merr.	Gogo	*b*	1	0.72
Erythrina variegata Linn. var. orientalis (Linn.) Merr.	Dapdap	*l*		0
Do	do	*s*		0
Do	do	*r*		0
Flemingia strobilifera (Linn.) R. Br.	Payang-payang	*l*		0
Do	do	*s*		0
Gliricidia sepium (Jacq.) Steud.	Kakawati	*l*		0
Do	do	*s*		0
Do	do	*b*		0
Indigofera suffruticosa Miller	Tayum	*l*		0
Do	do	*s*		0
Leucaena glauca (Linn.) Benth.	Ipil-ipil	*sd*		0
Mezoneurum latisiliquum (Cav.) Merr.	Dawag	*l*		0
Mezoneurum sumatranum (Roxb.) W. and A.	Siit	*l*		
Mimosa pudica Linn.	Makahiya	*l*		0
Do	do	*s*		0
Pachyrrhizus erosus (Linn.) Urb.	Sinkamas	*fr*		0
Do	do	*l*		0
Do	do	*s*		0
Do	do	*bu*	1	0
Parosela glandulosa (Blanco) Merr.	Sampaloc-sampaloc an	*s*		0
Do	do	*l*		0
Phaseolus aureus Roxb.	Mungo	*sd*		0
Phaseolus lunatus Linn.	Habichelas (red)	*sd*	1	0.30
Pithecolobium dulce (Roxb.) Benth.	Kamachile	*b*	1	0
Phaseolus lunatus Linn.	Habichuelas (white)	*sd*		0
Pongamia pinnata (Linn.) Merr.	Bani	*l*		0
Do	do	*s*		0
Psophocarpus tetragonolobus (Linn.)DC.	Seguidillas	*fr*		0
Pterocarpus indicus Willd.	Narra	*l*		0
Sesbania grandiflora (Linn.) Pers.	Katurai	*l*		0
Do	do	*s*		0

[1] Code for plant parts: *b*, bark; *bu*, bulb; *fr*, fruit; *l*, leaf; *r*, root; *rb*, root-bark; *rh*, rhizome; *s*, stem; *sd*, seed; *t*, tuber.

TABLE 1. *Results of hemolysis test and estimated sapogenin content found in the plant collections*—Continued.

Species	Local name	Plant part[1]	Hemolysis test	Estimated total m.f.b.
Do	do	*b*		0
Tamarindus indica Linn.	Sampaloc	*s,b*	1	0
Do	do	*l*		0
Vigna sesquipedalis Fruw.	Sitao	*sd*	1	0
Vigna sinensis (Linn.) Savi	Paayap	*sd*	1	0
APOCYNACEÆ				
Allamanda cathartica Linn.	Campanilla	*l*		0
Alstonia macrophylla Wall.	Batino	*l*		0
Do	do	*s*		0
Do	do	*b*	1	0.25
Do	do	*fr*		0
Catharanthus roseus (Linn.) Don	Chichirica	*l*		0
Do	do	*s*		0
Alstonia scholaris (Linn.) R. Br.	Dita	*l*		0
Do	do	*b*	1	0
Kibatalia blancoi (Rolfe) Merr.	Laniting-gubat	*l*	1	0.19
Do	do	*s*		0
Nerium indicum Mill.	Adelfa	*s*		0
Do	do	*l*		0
Do	do	*b*	1	0.98
Paralstonia clusiaceæ Baill.	Maladita	*b*	1	0
Plumiera acuminata Ait.	Kalachuche	*l*		0
Do	do	*b*	1	0.40
Tabernaemontana pandacaqui Poir.	Pandakaki	*l*		0
Thevetia peruviana (Pers.) Merr.	Campanero	*l*		0
Do	do	*s*	1	0
Rawolfia amsoniaefolia A. DC.	Sibakong	*rb*		0
DIOSCOREACEÆ				
Dioscorea alata Linn	Ubi	*t*	1	0.25
Do	do	*l*	1	0
Dioscorea esculenta (Lour.) Burkill	Tugi	*t*	1	0.65
Dioscorea hispida Dennst.	Nami	*t*	1	0.73
AMARYLLIDACEÆ				
Agave cantala Roxb.	Magei	*l*	1	0
Crinum latifolium Linn.	Lirio	*bu*		0
Eurycles amboinensis (Linn.) Lindl.	Cebollas del Monte	*bu*		0
Do	do	*l*		0
Hymenocallis littorale (Jacq.) Salisb.	Spider lily	*bu*		0
Do	do	*l*		0
LILIACEÆ				
Aloe vera Linn.	Sabila	*l*		0
Allium ascalonicum Linn.	Sibuyas tagalog	*bu*		0
Allium cepa Linn.	Sibuyas lumbay	*bu*		0
Allium odorum Linn.	Kuchai	*l*		0
Allium sativum Linn.	Bawang	*bu*		0
Asparagus plumosus Baker	Asparagus fern	*l*		0
Do	do	*fr*	1	0
Cordyline fruticosa (Linn.) A. Chev.	Sagilala	*l*		0
MALVACEÆ				
Abutilon indicum (Linn.) Sweet	Malbas	*l*		0
Do	do	*s*	1	0
Do	do	*fr*		0
Hisbiscus rosasinensis Linn.	Gumamela	*l*		0
Do	do	*s*		0
Sida acuta Burm. f.	Walliswalisan	*l*		0
Do	do	*s*		0
Do	do	*r*		0
Thespesia populnea (Linn.) Soland.	Bunlo	*r*	1	0
Do	do	*s*	1	0

TABLE 1. *Results of hemolysis test and estimated sapogenin content found in the plant collections*—Continued.

Species	Local name	Plant part[1]	Hemolysis test	Estimated total m.f.b.
EUPHORBIACEÆ				
Antidesma bunius (Linn.) Spr.	Bignai	*l*	1	0
Breynia rhamnoides (Retz.) Muell.-Arg.	Matang-hipon	*l*		0
Do	do	*s*		0
Euphorbia neriifolia Linn.	Soro-soro	*l*		0
Do	do	*s*		0
Euphorbia pulcherrima Willd.	Pascua	*l*		0
Do	do	*s*		0
Euphorbia tirucalli Linn.	Suerda	*s*	1	0.10
Excoecaria agallocha Linn.	Buta-buta	*l*		0
Do	do	*s*		0
Jatropha curcas Linn.	Tubang-baked	*l*	1	0
Do	do	*s*	1	0.90
Do	do	*b*	1	0.15
Macaranga tanarius (Linn.) Muell.-Arg.	Binoñga	*l*		0
Do	do	*s*	1	0
Do	do	*b*	1	0.96
Manihot esculenta Crantz.	Kamoteng-kahoy	*l*		0
Do	do	*l*		0
Do	do	*s*	1	0
Pedilanthus tithymaloides (Linn.) Poit.	Luhang-dalaga	*l*		0
Do	do	*s*		0
Ricinus communis Linn.	Tañgan-tañgan	*l*		0
Do	do	*s*	1	0.51
Do	do	*sd*		0

The sample, preferably 5.0 mg, was weighed into a 10-ml volumetric flask and dissolved in chloroform. The solvent was evaporated to dryness. Sulfuric acid, 94 per cent by volume, was added to the 10-ml mark. The flask was then immersed for 16 hours in a constant-temperature bath at 40°C. The flask and contents were then cooled to room temperature and the contents diluted, if necessary, to volume with 94 per cent sulfuric acid.

The sapogenins in the sample were determined qualitatively by the use of the Beckman DU spectrophotometer.

RESULTS AND DISCUSSIONS

The results obtained from one hundred thirty-nine extracts prepared from different parts of eighty-three local plants investigated are shown in Table 1. Forty-five alcoholic extracts were positive in the hemolysis test. Seven plants of the Leguminosæ family, 4 plants of the Apocynaceæ, 3 of the Dioscoreaceæ, and 5 of the Euphorbiaceæ, gave positive results in the spectrophotometric determination. Plant samples under the families Liliaceæ, Amaryllidaceæ, and Malvaceæ were found to be negative.

The ultraviolet spectrum of the solution, relative to the pure solvent, was obtained from 220 to 400 mμ in a 1-mm cell and examined for the presence of the characteristic sapogenin absorption bands. Fig. 1 shows the absorption maxima of nami

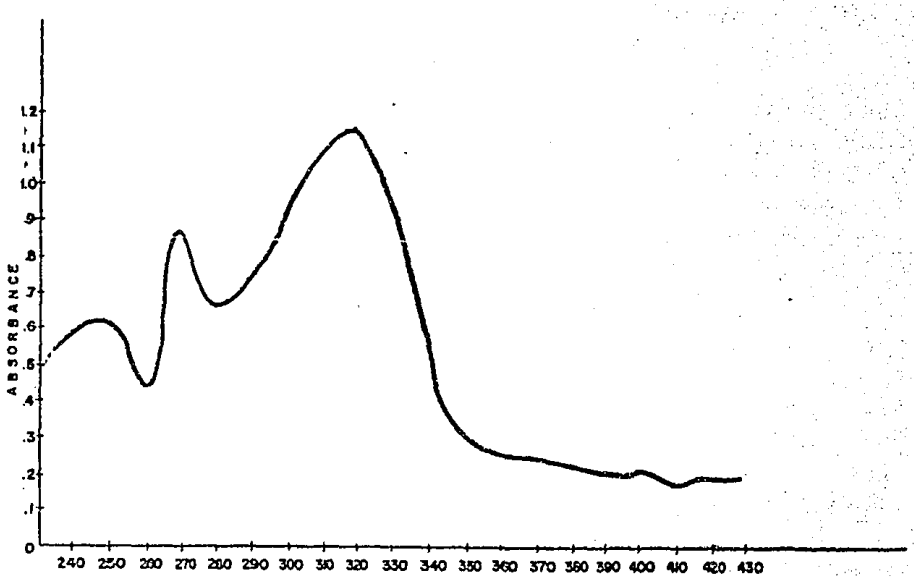

FIG. 1. Absorption spectra of *Dioscorea hispida* Dennst. (nami) tuber.

tuber at 250, 270, 320, 400, and 415 mμ. By comparison with the standard maxima given in Table 2, the sapogenin content of the solution can be qualitatively determined.

TABLE 2.—*Wave length positions and intensities of absorption maxima of sulfuric acid chromogens of steroidal sapogenins.*

Sapogenin	Absorption maxima, Mμ
Chlorogenin	270,330,415
Diosgenin	271,415,514
Gitogenin	272,308
Hecogenin	276,350,396
Kammogenin	233,272,349
Kryptogenin	280,383
Manogenin	276,348,400,468
Markogenin	270,308
Rockogenin	273,379
Samogenin	270,308
Sarsasapogenin	271,310
Smilagenin	272,312
Tigogenin	270,312
Yuccagenin	240,268,405

For an approximate determination of the quantity of sapogenin in the sample, it is measured at 250 m using the absorption coefficients given in Table 3. The concentration of sapogenin

TABLE 3.—*Absorptivities of steroidal sapogenins at 250 and 350 mμ.*[a]

Sapogenin	Absorption coefficients 250 mμ	350 mμ
Chlorogenin	16.0	18.4
Diosgenin	18.4	14.6
Gitogenin	18.1	8.8
Hecogenin	16.3	29.6
Kammogenin	18.3	17.5
Kryptogenin	9.9	12.3
Manogenin	15.3	23.3
Markogenin	16.6	10.9
Rockogenin	17.9	22.4
Sammogenin	17.2	12.0
Sarsasapogenin	15.3	13.9
Smilagenin	16.4	13.9
Tigogenin	15.7	13.9
Yuccagenin	28.0	12.4

[a] Absorbtivity is defined as $a = A/bc$ where A is the absorbance of a solution of thickness b (centimeters) and c grams per liter compared with an equal thickness of solvent.

was computed by the following formula adopted by Wall, et al.:

$$c = \frac{A}{ab}$$

where,

a = absorptivity coefficient
A = absorbance
b = thickness of cell in cm
c = concentration of sapogenin in grams/liter

In the case of samples that contain mixtures of steroidal sapogenins the absorption coefficients of which are quite far apart, estimation is not satisfactory without preliminary separation into individual sapogenins. However, from the spectral curves taken, conclusions can be drawn whether such samples contain appreciable amounts of steroidal sapogenins.

SUMMARY

Different parts of eighty-three local plant materials belonging to the families Leguminosæ, Apocynaceæ, Dioscoreceæ, Amaryllidaceæ, Liliaceæ, Malvaceæ, and Euphorbiaceæ were extracted with 80 per cent ethyl alcohol. One hundred thirty-nine extracts

were obtained and all were subjected to hemolysis test. Forty-five alcoholic extracts gave positive results. Positive samples were isolated for their crude sapogenin acetates. Adsorption chromatography using activated alumina as the adsorbing agent removed substances or resins which would react with the sulfuric acid remaining in the sample. At the same time, it separated the monohydroxy from the dihydroxy sapogenins which made identification of steroidal sapogenins easier. Nineteen out of the forty-five that were isolated gave steroidal sapogenin spectral curves in the region of 220 to 400 mμ. Some of these are the barks of *Nerium indicum* Mill. (adelfa), *Jatropha curcas* Linn. (tubang-bakod), *Macaranga tanarius* (Linn.) Muell.-Arg. (binoñga), and the tubers of *Dioscorea hispida* Dennst. (nami). Plant samples investigated under the families Liliaceæ, Amaryllidaceæ, and Malvaceæ were found to contain no steroidal sapogenins.

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