Medicinal Folklore Evaluation I

Alleged Androgenic and Aphrodisiac Action of Pega Palo (*Rhynchosia pyramidalis*)

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Pega palo is a plant that has received recent and widespread publicity as an alleged aphrodisiac and androgenic agent. One literature report claims clinical efficacy for these effects; however, other investigators could not confirm these activities in laboratory animals. In the present investigation, using a variety of biological test procedures, neither androgenic activity nor an aphrodisiac effect could be demonstrated in laboratory animals.

RHYNCHOSIA PYRAMIDALIS (Lam.) Urb. (*Leguminosae*), is a woody vine found extensively in the Dominican Republic and in Haiti. Dominican folklore and tradition, dating to pre-Columbian times, reveal that alcoholic extracts prepared from the stems of this plant have been used empirically for the purpose of increasing libido and strengthening of sexual performance in the male (1–3). More recently, several popular magazines and newspapers have reported on the more sensational aspects of the biological activity associated with *R. pyramidalis* (2). The use of pega palo (virility vine) in the United States for these purposes has been widely publicized, and its availability to the general public advertized in newspapers and magazines. Since labeling of such material failed to indicate adequate directions for use, and since scientific evidence establishing any specific biological activity was not available, and because new drug applications had not been filed, both the Federal Food and Drug Administration and the U. S. Post Office Department have prevented further distribution of pega palo through interstate commerce (1).

Objective evidence concerning the alleged aphrodisiac and/or androgenic effect of *R. pyramidalis* is restricted to two reports. In 1960, Danowski and Price (4) determined that the inclusion of 5% *R. pyramidalis* in the diet of normal and hypophysectomized rats for 26 and 56 days, respectively, resulted in observations of no appreciable effect on the testes, prostate, or seminal vesicles of treated animals. On the other hand, Sobá *et al.* (3) reported that in a controlled clinical study, which involved 50 cases of sexual impotence, an androgenic effect was observed in 82% of the patients receiving a pega palo tincture.

Folkers and Unna (6) have reported that in old seeds of *R. pyramidalis*, traces of alkaloids could be detected. Furthermore, they indicated that extracts from these seeds possessed some curare-like activity. More recently, Ristić and Thomas (7) have isolated an alkaloid (picrate, m.p. 132°), which by thin-layer chromatography was shown to be different from yohimbine. Santesson (8) examined the closely related *Rhynchosia phaseoloides* and found it to contain alkaloids and glucosides, but further indicated that it was devoid of curare-like activity in the frog. No other reports in the literature have been found concerning the phytoconstituents of *Rhynchosia* species.

The controversy in the literature regarding the biological activity of *R. pyramidalis*, together with a desire to explore the value of this plant as a source of new medicinal agents, prompted the evaluation of extracts of the vine of *R. pyramidalis* through a variety of biological screening tests.

EXPERIMENTAL

Materials—The plant material used in this investigation was the vine of *R. pyramidalis* (Lam.) Urb. (*Leguminosae*), collected in Haiti during September of 1961 and supplied by S. B. Penick & Co., New York, N. Y. A voucher specimen has been deposited there.

Preparation of Extracts for Biological Testing—A defatted ethanol extract was utilized for the antimicrobial test, the antitumor test, and for the mouse behavior evaluation. The method for preparing this extract has been previously described (5).

According to Sobá *et al.* (3), the native custom in Haiti for preparing pega palo tincture is to steep the dried vine in rum for several days. In the clinical studies conducted by this group, a 15% "hydro-alcoholic" extract of the vine was used (5).[1] Extracts utilized in the present investigation

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[1] Identified as Tintura Pega Palo Fortidum, Laboratorio Quimico Dominicano, Ciudad Trujillo, D.N., Dominican Republic.

for the androgenic activity assays, and for the sexual behavior studies were prepared in the following manner.

In a typical extraction, 125 Gm. of coarsely powdered vine was moistened with 50% (w/v) ethanol and allowed to stand for 1 hr. The moistened drug was then packed into a suitable cylindrical glass percolator, sufficient 50% ethanol was added to cover the drug, and the mixture was allowed to macerate for 24 hr. Percolation was then initiated at a moderate rate until 1000 ml. of percolate was collected. The tincture prepared in this manner was then reduced to a volume of 250 ml. using a flash evaporator (*in vacuo*, 40°), thus removing any ethanol that might alter the biological activity of active constituents in the vine when evaluated in animals. A cloudy aqueous suspension resulted, which was of high viscosity and required no suspending agent. This extract, which contained the extractives of 0.5 Gm. of vine in each 1.0 ml., was labeled extract *A*, and was used in all evaluations requiring an oral route of administration. In experiments involving intraperitoneal administration of the extract, a less concentrated aqueous suspension was prepared by dilution with water so that each 1.0 ml. was equivalent to the extractives from 0.13 Gm. of vine (extract *B*).

General Biological Evaluation—Using methods previously described for screening native plants (5), a defatted ethanol extract of *R. pyramidalis* vine was evaluated for activity against 16 bacterial animal pathogens and saprophytes, three fungal animal pathogens and saprophytes, four bacterial plant pathogens, 15 fungal plant pathogens and material degrading organisms, and three protozoa. Using the agar diffusion technique, the extract was found to be inhibitory for *Mycobacterium avium* and *Lactobacillus casei* at the 100 mcg./ml. level. No activity was demonstrated against any of the other test organisms.

Similarly, the extract was devoid of activity against the C-1498 leukemia, P-1534 leukemia, adenocarcinoma 755, mecca lymphosarcoma, and the X-5563 myeloma, all in mice (5). In the general mouse behavior evaluation (5), the defatted ethanol extract was classified as having weak central nervous system depressant properties.

Assay for Androgenic Activity—Extract *B* (0.13 Gm./ml.) was administered i.p. daily to each of six albino Wistar male rats, previously castrated in the usual manner. Injections were initiated 24 hr. following the surgical procedure, and continued for nine successive days at a dose of 5.0 ml./Kg. (0.65 Gm./Kg.). No unusual effects were noted in the treated animals during this period of time. A second group of six rats were similarly castrated and received daily i.p. injections (5.0 ml./Kg.) of normal saline solution. Twenty-four hours following the final injection, all animals were sacrificed, the seminal vesicles and prostate gland removed from each animal, blotted between paper to remove seminal fluid and excess moisture, and carefully weighed.

The androgenic activity of extracts can be ascertained by noting the lack of any significant increment in relative weights of the prostate and seminal vesicles over saline-treated controls. The results are presented in Table I.

Following autopsy of the animals, it was observed that the peritoneum of each extract-treated rat was colored dark brown. The absence of androgenic activity, together with this observation, presented the possibility of hindered absorption of active principles in the extract due to the presence of tannins. In addition, Sobá *et al.* (3) have reported that administration of pega palo tincture to humans over extended periods of time usually gave more satisfactory results. This seemed to apply in this study as an additional explanation for the negative results, since nine doses could have been inadequate. The authors therefore modified the initial androgenic assay by: (*a*) utilizing a more concentrated extract (extract *A*), (*b*) administering the extract at three different dosage levels (5.0 ml./Kg., 10.0 ml./Kg., and 20.0 ml./Kg.), (*c*) giving the extract orally by means of a suitable stomach tube, and (*d*) continuing the extract administration for a longer period of time (single daily doses for 79 successive days).

TABLE I—EFFECT OF *R. pyramidalis* EXTRACT *B* ON THE WEIGHT OF CASTRATE RAT SEMINAL VESICLES AND PROSTATE GLAND

Animal	Dose,[a] ml./Kg.	Type Extract	Prostate Wt., mg./100 Gm. Body Wt.	Seminal Vesicles Wt., mg./100 Gm. Body Wt.	Body Wt. at Autopsy
1	5.0	*B*[b]	35	32	177
2	5.0	*B*	22	33	160
3	5.0	*B*	37	37	200
4	5.0	*B*	29	36	171
5	5.0	*B*	24	37	178
			—	—	—
			$\bar{x}$ 31	35	178
7	5.0	Saline	31	36	195
8	5.0	Saline	34	38	175
9	5.0	Saline	41	37	163
10	5.0	Saline	45	38	183
11	5.0	Saline	21	33	174
12	5.0	Saline	28	33	162
			—	—	—
			$\bar{x}$ 33	36	175

[a] Nine successive daily i.p. injections were made. [b] Extract *B* contained the extractives from 0.13 Gm. of vine in each 1.0 ml.

The results of this study, utilizing extract *A* (0.5 Gm./ml.) are presented in Table II and substantiate the negative findings of the initial study. It was further observed that no unusual gross effects were seen in the drug-treated animals during the 79 days of the study.

Representative tissue specimens from the prostate, seminal vesicles, penis, bladder, kidney, and liver were removed from several drug-treated and control rats at autopsy and these were examined for evidence of pathological or abnormal effects. No evidence of abnormal tissue was observed in the specimens examined.

Effect of Extract A on the Sexual Behavior of Normal Rats—Twenty hooded male rats, 120–150 days of age, were randomly assigned to experimental and control groups. Following a series of five 1 hr.–once weekly copulation tests with receptive females, the drug phase of the study was initiated. Experimental rats received extract *A* daily by stomach tube (4.4 ml./Kg., equivalent to 2.2 Gm./Kg.), while controls were given an equivalent volume of water by stomach tube. Weekly, hour-long copulation tests with receptive females were continued for 20 weeks, with the 10th and 15th test following initiation of drug administration being

TABLE II—EFFECT OF *R. pyramidalis* EXTRACT *A* ON THE WEIGHT OF CASTRATE RAT SEMINAL VESICLES AND PROSTATE GLAND

Animal	Dose,[a] ml./Kg.	Type Extract[b]		Prostate Wt., mg./100 Gm. Body Wt.	Seminal Vesicles Wt., mg./100 Gm. Body Wt.	Body Wt. at Autopsy
1	5.0	*A*		222	95	413
2	5.0	*A*		178	107	486
3	5.0	*A*		137	84	365
4	5.0	*A*		153	95	353
			$\bar{x}$	173	95	404
5	10.0	*A*		217	89	487
6	10.0	*A*		187	73	380
7	10.0	*A*		125	101	417
8	10.0	*A*		233	121	393
			$\bar{x}$	191	96	419
9	20.0	*A*		175	101	424
10	20.0	*A*		165	94	435
11	20.0	*A*		163	101	418
12	20.0	*A*		265	95	457
13	20.0	*A*		165	65	393
14	20.0	*A*		261	100	361
			$\bar{x}$	199	93	415
15	10.0	Water		180	100	428
16	10.0	Water		237	107	443
17	10.0	Water		153	78	405
18	10.0	Water		148	116	355
19	10.0	Water		177	105	360
			$\bar{x}$	179	101	398

[a] Seventy-nine successive single oral daily doses. [b] Extract *A* contained the equivalent of 0.5 Gm. of extractives in each 1.0 ml.

satiation tests. Satiation tests have no specific time limit, but continue until the male spends 30 min. without making a sexual response. During the time course of the experiment, males were kept in isolation except during the actual sexual tests.

Measures taken during sexual behavior tests included latency to first mount, number of incomplete mounts (no intromission), number of complete mounts (intromissions), number of ejaculations, length of refractory periods (time from ejaculation to the next sexual response), and amount of time spent in actual sexual behavior. For all of the measures taken, *t* tests were run between experimental and control groups with separate tests run for the pre-drug tests and the total post-drug tests. Also, in case the results of drug administration were cumulative, or gradual in onset, post-drug tests were split into first 10 test and last 10 test categories. These data were then compared within and between control and experimental groups.

No significant ratios were found for any of the above measures or comparisons. It is therefore concluded that the administration of *R. pyramidalis* extract *A* to male rats under these conditions, and at the dosage level indicated, has no significant effect on any of the sexual parameters measured.

Phytochemical Screening—Using the methods of Wall *et al.* (9–11), *R. pyramidalis* vine was found to contain trace amounts of alkaloids, tannins of the catechin type, saponins, and organic acids and phenols. Flavonoids could not be detected using these methods.

Examination of R. pyramidalis Alkaloids—Using the U.S.P. assay for belladonna alkaloids, *R. pyramidalis* vine was found to contain 0.19% of total crude alkaloids (as strychnine) (12).

A thin-layer chromatogram of the total crude alkaloids from *R. pyramidalis* vine was prepared as follows. One hundred grams of powdered vine was moistened with 28% NH_4OH, air-dried, and continuously extracted with chloroform. The chloroform extract was concentrated to 10.0 ml. Twenty microliters was applied to a Silica Gel G plate (0.25 mm.), prepared in the usual manner, and eluted with methanol. After air-drying the plate, it was sprayed with Dragendorff's reagent. Three typical orange-colored alkaloid spots were noted at R_f 0.08, 0.22, and 0.35.

DISCUSSION

The results of this study clearly indicate that the *R. pyramidalis* extracts utilized were devoid of androgenic activity as evidenced by an absence of accessory organ stimulation in castrate rats. These findings are in agreement with those of Danowski and Price (4), who also used rats as assay animals. However, both our results and those of Danowski and Price are contradictory to those reported by Sobá *et al.* (3), who evaluated pega palo tincture in 50 clinical cases of sexual impotence with partial or complete loss of libido and erections. On the other hand, the paper by Sobá *et al.* (3) is entitled "Androgenic Action of Pega Palo," but the biological activity described in this paper alluded to a restoration of libido and erections. These workers performed no assays to indicate that their extracts contained truly androgenic materials. Furthermore, although the authors mention that, "For comparison, in many cases, a placebo was substituted for the Pega Palo," (3), no details are given concerning the type of placebo used, or the results obtained with the placebo preparation. In addition, it was reported that 50 cases of sexual impotence with partial or complete loss of libido and erections were included in the study, and their conclusions indicated that 41 patients were restored to normal activity during the period of medication, whereas nine (making a total of 50) showed no increase in libido or erectile power. If these 50 patients constituted the entire group studied, and no statements to the contrary were made, it is difficult to ascertain how a placebo preparation was used for comparison.

When plant material is being evaluated for biological activity, it is often difficult to make valid comparisons between the results obtained in any two different laboratories, even under ideal conditions. One must consider not only variations in the test methods but also variability in the chemical constituents of investigational plant material due to ecological factors, the age of the plant at harvest, methods of processing the collected specimens, and the possibility of working with chemical races of the same species. Further complicating factors, which appear to apply in the present situation, are the possibility of improper identification of the material being investigated, as well as the problem of selecting the correct plant material when reference is made only to the vernacular name.[2]

Danowski and Price (4) in their studies utilized a sample of plant material identified only as "Pega Palo." An authentic voucher specimen of their

[2] Sobá *et al.* (3) have pointed out that two unrelated low shrubs are also sometimes referred to by the vernacular names "pega pallo" and "pega palo," but these resemble *R. pyramidalis* neither in appearance nor herbal properties.

material was not made (13). Thus the distinct possibility exists that their plant material was not, in reality, *R. pyramidalis*. Along similar lines, no mention was made in the paper of Sobá *et al.* (3) that they deposited a voucher specimen representative of their investigational material for reference purposes.

With regard to chemical variability, the authenticated material gave definite positive tests for alkaloids (*vide supra*), which has been duplicated and confirmed by the studies of Ristić and Thomas (7). The material studied by Sobá *et al.* (3) was devoid of alkaloids, although their test methods were not defined.

SUMMARY

The vine of *R. pyramidalis* (Lam.) Urb. (*Leguminosae*), popularly known as pega palo and used in folkloric medicine as an aphrodisiac, has been the subject of the present investigation. Extracts from this plant were found to be devoid of androgenic activity and did not significantly alter the sexual behavior of normal rats when the following measures were taken: latency to first mount, number of incomplete mounts (no intromission), number of complete mounts (intromission), number of ejaculations, length of refractory periods (time from ejaculation to the next sexual response), and amount of time spent in actual sexual behavior.

In a general biological screening of a defatted ethanol extract of *R. pyramidalis* vine, inhibitory activity was observed against *Mycobacterium avium* and *L. casei* (*in vitro*). The extract was devoid of antitumor activity, and in gross observation studies using mice, central nervous system depression was observed.

The vine was found to contain three alkaloids by means of thin-layer chromatography, comprising 0.19% of total crude bases. Tannins, saponins, and organic acids and phenols were also found to be present, but tests for the presence of flavonoids were negative.

REFERENCES

(1) Anon., *J. Am. Med. Assoc.*, **165**, 695(1957).
(2) Landman, D., *True, The Man's Magazine*, **39**, 46 (1958).
(3) Sobá, J. G., Molano, E. E. S., and Damrau, F., *J. Natl. Med. Assoc.*, **52**, 25(1960).
(4) Danowski, T. S., and Price, L., *Endocrinology*, **66**, 788(1960).
(5) Farnsworth, N. R., Henry, L. K., Svoboda, G. H., Blomster, R. N., Yates, M. J., and Euler, K. L., *Lloydia*, **29**, 101(1966).
(6) Folkers, K., and Unna, K., *J. Am. Pharm. Assoc., Sci. Ed.*, **28**, 1019(1939).
(7) Ristić, S., and Thomas, A., *Arch. Pharm.*, **295**, 510 (1962).
(8) Santesson, C. G., *Etnologiska Studier*, **6**, 179(1938).
(9) Wall, M. E., Krider, M. M., Krewson, C. F., Eddy, C. R., Willaman, J. J., Correll, D. S., and Gentry, H. S., *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 1(1954).
(10) Wall, M. E., Fenske, C. S., Garvin, J. W., Willaman, J. J., Jones, Q., Schubert, B. G., and Gentry, H. S., *ibid.*, **48**, 695(1959).
(11) Wall, M. E., Eddy, C. R., McLennab, M. L., and Klumpp, M. E., *Anal. Chem.*, **243**, 1337(1952).
(12) "United States Pharmacopeia," 17th rev., Mack Publishing Co., Easton, Pa., 1965, p. 62.
(13) Danowski, T. S., School of Medicine, University of Pittsburgh, Pittsburgh, Pa., personal communication.

Trifluoromethyl Analogs of Amphetamine and Norephedrine

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Trifluoromethyl analogs of amphetamine (I) and norephedrine (II) were synthesized for pharmacological evaluation. They showed no activity in profile tests for anorectic activity (I), antiemetic activity (I), CNS activity (I), MAO-inhibitory activity (I, II), and pressor activity (II).

THE SYNTHESIS and pharmacological investigation of organic fluorine compounds has led to the development of such important drugs as the fluorinated inhalation anesthetics (1), the fluorinated corticosteroids (2), the trifluoromethylphenothiazine tranquilizers and the trifluoromethylthiazide diuretics (1, 2). A number of compounds containing the trifluoromethyl group have been found to possess anesthetic, ataractic, and antiemetic (1), diuretic (1, 3), antihistaminic (4), tumor inhibitory (5, 6), and antimicrobial (7, 8), hypotensive (9), and spasmolytic (10) properties.

The trifluoromethyl group is particularly suited to replace the methyl group in known pharmacologically active compounds, both because of the similarity in physical properties of the two classes of compounds and because of the unique chemical and physiological stability of the trifluoromethyl group. The pronounced electron-withdrawing properties of the group will also be expected to play a part in modifying the activity of the prototype compounds.

The authors have now carried out a synthesis of 2-amino-3-phenyl-1,1,1-trifluoropropane (I),

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