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Final Report

Contract No. RFP - 68

DA 18-108-CML-6613

CPI-15190

From: June 1, 1961 to June 1, 1962

TITLE OF PROJECT: "The Collection and Study of Plants Containing
Disabeling Agents."

FROM: Department of Pharmacology and
The Brain Research Institute, School of Medicine
and
Department of Botany, University of California,
Los Angeles

PRINCIPAL INVESTIGATORS: Dermot B. Taylor, Professor and Chairman
Department of Pharmacology and Member of
the Brain Research Institute, University
of California Medical Center, Los Angeles
24, California

and

John A. Bevan, Associate Professor of
Pharmacology, University of California
Medical Center, Los Angeles 24, California

Signed: _____

Dermot B. Taylor, M.A., M.D.

Summary

(1) Previously all plants collected were obtained by summer expeditions from the U.S.A. During the past year a Peruvian working for us has been sending substantial quantities of plants throughout the year and the lists of these are attached.

(2) A summary of our progress in developing methods for grinding, extracting and screening plants is attached.

(3) The results of our efforts to screen for plants with actions on the central nervous system are attached.

A. Personnel working on the contract:

(a) Receiving whole-time or part-time salary from
the contract:

I. Mildred E. Mathias, Ph.D.

Professor of Botany and

Director of Botanical Gardens

II. Paul Hardy, Ph.D.

Assistant Research Pharmacologist

III. Elizabeth Lomax, M.D.

Graduate Research Pharmacologist

IV. A considerable number of local people hired
from time to time in Peru to assist in a
variety of tasks.

(b) Working without payment from the contract:

I. Dermot B. Taylor, M.A., M.D.

II. John A. Bevan, M.B., B.S.

III. Charles Spooner, A.B.

B. Plant Collections.

The most significant development during the past year was the employment of Mr. Jose Schunke on a full-time basis to collect plants in Peru between our annual expeditions. Mr. Schunke has now received extensive training from our Dr. Mildred Mathias in botany and plant collection.

The collection of plants all year has several distinct advantages over the summer expeditions to which we confined our efforts previously. It enabled us to obtain previously tested and new plants when they were in flower. In many cases flowers are necessary for identification. In addition this arrangement improves the supply of interesting plants and costs somewhat less.

Numbers of Plants Received from Peru.

During the period between January 1962 to May 1962, Mr. Schunke sent us some hundred and seventy analysable plant samples.

Lista de Plantas del Peru

Dept. de San Martin Distrito de Uchiza

Enero y Febrero de 1962

Plant No.

- 5732 Sapindaceae, arbusto; (2) paquetes Trunk, fruit y herbario.
- 5733 Rubiaceae arbusto; solo herbario.
- 5734 Rubiaceae arbusto; (1) paquete trunk y herbario.
- 5735 Arbol.- Utilizan latex mixto con la flor del clavel para enfermedad de epilepsia; bebiendo un vaso de infussion al acostarse en las noches, (1) bolsa de bark sin herbario.
- 5736 Rubiaceae arbusto (1) paquete trunk y herbario
- 5737 Paujil Huasca (1) paquete trunk y herbario.
- 5738 Anonaceae arbusto (1) paquete trunk y herbario.
- 5739 Sapindaceae Liana (2) paquetes trunk fruit y herbario.
- 5740 Liana solo herbario.
- 5741 Solanaceae solo herbario.
- 5742 Rubiaceae arbusto (1) paquete trunk y herbario.
- 5743 Bignoneaceae arbusto; utilizan zumo de leaves en cocimiento para curar llagas infecciosos (2) leaves, trunk y herbario.
- 5744 Bignoneaceae liana, solo herbario.
- 5745 Orchidaceae solo herbario.
- 5746 Liana (1) paquete trunk y herbario.
- 5747 Cucurbitaceae solo herbario.
- 5748 Arbusto (1) paquete trunk y herbario
- 5749 Cucurbitaceae solo herbario.
- 5750 Arbusto.- Bark utilizan en maceracion para enfermedad de disenteria bebiendo la cuarta parte de un vaso (1) bolsa de bark y herbario.
- 5751 Bignoneaceae-liana (1) paquete trunk y herbario.
- 5752 Solanaceae arbusto, utilizan leaves en infusion para lavar heridas infecciosas; (3) leaves, trunk, fruit y herbario.
- 5753 Arbusto (1) bolsa fruit y herbario.
- 5754 Anonaceae arbusto solo herbario.
- 5755 Liana (2) root, trunk y herbario.
- 5756 Arbusto solo herbario.
- 5757 Arbusto solo herbario.
- 5758 Rubiaceae arbusto (1) paquete trunk y herbario.
- 5759 Arbusto.- Utilizan bark en maceracion mixto con aguardiente para enfermedad de paludismo; bebiendo en las manas, este mismo utilizan para curar asma (1) paquete trunk y herbario.
- 5760 Arbusto.- Utilizan fruit como laxante (1) paquete trunk y herbario.
- 5761 Hierba solo herbario.

Plant No.

- 5762 Arbusto (1) paquete trunk y herbario.
5763 Arbusto solo herbario.
5764 Cucurbitaceae solo herbario.
5765 Arbol (2) paquetes bark, fruit y herbario.
5766 Rubiaceae arbusto (2) trunk, root y herbario.
5767 Arbusto solo herbario.
5768 Rubiaceae arbusto (1) paquete trunk y herbario.
5769 Arbusto (1) paquete trunk y herbario.
5770 Apocynaceae arbusto (2) root, bark y herbario.
5771 Rubiaceae arbusto (1) paquete trunk y herbario.
5772 Sapindaceae, liana (2) trunk, fruit y herbario.
5773 Loranthaceae (1) bolsa y herbario.
5774 Arbusto (1) paquete trunk y herbario.
5775 Passifloraceae.-liana (2) root, trunk y herbario.
5776 Apocynaceae solo herbario.
5777 Sapindaceae liana (1) paquete trunk y herbario.
5778 Rubiaceae solo herbario.
5779 Hierba solo herbario.
5780 Bignoneaceae liana (1) paquete trunk y herbario.
5781 Leguminosae arbusto (1) paquete trunk y herbario.
5782 Hongos solo herbario.
5783 Leguminosae arbol (1) bolsa bark y herbario.
5784 Apocynaceae solo herbario.
5785 Arbusto.-Utilizan leaves para depurar la sangre (1) paquete trunk y herbario.
5786 Hierba. Utilizan, rizoma en cocimiento para facilitar el parto; tambien la misma rizoma utilizan para preservar de la picadura de culebra; solamente llevando en el bolsillo (1) bolsa y herbario.
5787 Rubiaceae liana.- Los brujos dicen que utilizan para mesclar el Aya-Huasca en cocimiento (1) paquete trunk y herbario.
5788 Arbol (2) trunk, fruit y herbario.
5789 Rubiaceae liana solo herbario.
5790 Sapindaceae arbusto solo herbario.
5791 Arbusto (1) paquete trunk y herbario.
5792 Hierba solo herbario.
5793 Arbusto (1) paquete, trunk y herbario.
5794 Arbusto (1) paquete trunk y herbario.
5795 Hierba solo herbario.
5796 Rubiaceae arbusto (1) paquete trunk y herbario.
5797 Arbusto solo herbario.
5798 Passifloraceae solo herbario.
5799 Arbusto (1) paquete trunk y herbario.
5800 Apocynaceae arbusto (1) trunk y herbario.
5801 Melastomaceae hierba solo herbario.
5802 Rubiaceae arbusto (1) paquete trunk y herbario.

Lista de Plantas del Peru

Departamento de Huanuco y Loreto

Marzo, Abril y Mayo de 1962.

Plants received 18 June, 1962

Plant No.

- 5803 Arbusto, solo herbario.
5804 Cucurbitaceae hierba, solo herbario.
5805 Passifloraceae.- Utilizan leaves en infusion para depurar la sangre (2) paquetes trunk, fruit y herbario.
5806 Arbusto (2) trunk, fruit y herbario.
5807 Arbusto (1) paquete, trunk y herbario.
5808 Liana Murcu Huasca (1) paquete trunk y herbario.
5809 Sapindaceae arbusto (1) paquete trunk y herbario.
5810 Myrtaceae, arbusto (2) paquetes trunk; fruit y herbario.
5811 Arbusto (1) paquete trunk y herbario.
5812 Leguminosae arbusto (2) fruit trunk y herbario.
5813 Bignoniaceae liana (1) paquete trunk y herbario.
5814 Piperaceae arbusto (2) leaves, trunk y herbario.
5815 Loranthaceae hirba (2) trunk, leaves y herbario.
5816 Loranthaceae hirba (1) leaves y herbario.
5817 Piperaceae arbusto (2) trunk, leaves y herbario.
5818 Arbusto (1) paquete trunk y herbario.
5819 Begoniaceae solo herbario.
5820 Arbusto (1) paquete trunk y herbario.
5821 Arbusto (1) paquete trunk y herbario.
5822 Cucurbitaceae liana (1) paquete trunk y herbario.
5823 Rubiaceae arbusto (1) paquete trunk y herbario.
5824 Sapindaceae arbusto (1) paquete trunk y herbario.
5825 Liana (2) trunk, fruit y herbario.
5826 Apocynaceae solo herbario.
5827 Rubiaceae arbusto (1) paquete trunk y herbario.
5828 Annonaceae arbusto (1) paquete trunk y herbario.
5829 Arbusto (1) paquete trunk y herbario.
5830 Moraceae arbol (3) bolsas, bark, fruit y herbario.
5831 Rubiaceae arbusto (1) paquete trunk y herbario.
5832 Arbusto (1) paquete trunk y herbario.
5833 Arbusto (1) paquete trunk y herbario.
5834 Hierba solo herbario.
5835 Hongos solo herbario.
5836 Hierba solo herbario.
5837 Liana (1) paquete trunk y herbario.
5838 Hierba solo herbario.
5839 Sapindaceae liana (2) paquetes trunk, fruit y herbario.
5840 Malpighiaceae liana (1) paquete trunk y herbario.
5841 Arbusto (1) paquete trunk y herbario.
5842 Murco Huasca liana (1) paquete trunk y herbario.

Plant No.

5843	Hierba solo herbario.
5844	Arbusto (1) paquete trunk y herbario.
5845	Arbusto solo herbario.
5846	Liana (1) paquete trunk y herbario.
5847	Arbusto (2) trunk fruit y herbario.
5848	Seadotenia Toxifera (1) paquete trunk y herbario.
5849	Seadotenia Toxifera (1) paquete trunk y herbario.
5850	Seadotenia Toxifera (1) paquete trunk y herbario.
5851	Seadotenia Toxifera (2) paquetes trunk y herbario.
5852	Arbusto (1) paquete trunk y herbario.
5853	Bignoniaceae Liana. Utilizan en infusion para purgante de los perros (1) paquete trunk y herbario.
5854	Arbusto (1) paquete trunk y herbario.
5855	Arbusto (1) paquete trunk y herbario.
5856	Arbusto (1) paquete trunk y herbario.
5857	Hierba solo herbario.
5858	Dioscoreaceae hierba solo herbario.
5859	Arbol (1) paquete trunk y herbario.
5860	Arbol (1) paquete trunk y herbario.
5861	Menispermaceae liana (1) paquete trunk y herbario.
5862	Arbusto solo herbario.
5863	Arbusto (1) paquete trunk y herbario.
5864	Cucurbitaceae hierba solo herbario.
5865	Begoniaceae hierba solo herbario.
5866	Solanaceae arbusto. Leaves utilizan en infusion para poner enema por enfermedad de gripe (1) bolsa, leaves y herbario.
5867	Rubiaceae arbusto (1) paquete trunk y herbario.
5868	Liana (1) paquete trunk y herbario.
5869	Rubiaceae arbusto (1) paquete trunk y herbario.
5870	Hierba solo herbario.
5871	Hierba solo herbario.
5872	Sapindaceae liana (1) paquete trunk y herbario.
5873	Hierba solo herbario.
5874	Melastomaceae arbusto solo herbario.
5875	Leguminosae liana (1) paquete trunk y herbario.
5876	Arbusto (1) paquete trunk y herbario.
5877	Arbusto solo herbario.
5878	Liana (1) paquete trunk y herbario.
5879	Liana (1) paquete trunk y herbario.
5880	Arbusto (1) paquete trunk y herbario.
5881	Arbusto (1) paquete trunk y herbario.
5882	Malpighiaceae liana (1) paquete trunk y herbario.
5883	Hierba solo herbario.
5884	Arbusto solo herbario.
5885	Hierba solo herbario.
5886	Menispermaceae liana solo herbario.
5887	Arbusto (1) paquete trunk y herbario.
5888	Murco Huasca liana (1) paquete trunk y herbario.

Plant No.

5889 Annonaceae arbusto (1) paquete trunk y herbario.
5890 Arbusto solo herbario.
5891 Arbusto. Utilizan leaves en infusion para dolor de
cabeza; y tambien el Zumo sirbe para mesclar curare en
cocimiento (1) bolsa leaves y herbario.
5892 Hierba (1) bolsa leaves y herbario.
5893 Hierba solo herbario
5894 Liana solo herbario.
5895 Leguminosae liana (1) paquete trunk y herbario.
5896 Arbusto (1) paquete trunk y herbario.
5897 Arbusto (1) paquete trunk y herbario.
5898 Leguminosae liana (1) paquete trunk y herbario.
5899 Bignoniaceae liana (1) paquete trunk y herbario.
5900 Hierba solo herbario.
5901 Arbusto solo herbario.
5902 Leguminosae arbusto solo herbario.

Introduction

The solution to the problem of the preparation of extracts suitable for screening and submission of samples to the Army Chemical Center is crucial to the whole program. The following account is the summation of our experience so far in this field.

1. Grinding.

(a) Use of Fitzpatrick Mill.

In our experience the Fitz Mill is the most suitable machine for grinding a large quantity of dried plant material. However it has a serious disadvantage for the grinding of small samples of large numbers of plants because of the long time necessary for the cleaning of the machine after each sample. In our routine screening processes we have to grind several hundred different 50 gram samples. The total cleaning time in an operation such as this is uneconomical and an alternative has had to be developed.

(b) Use of Industrial Size Waring Blender.

The difficulty in using this machine is that a solvent has to be incorporated with the wood at the outset and not all solvents are suitable for this purpose for a number of reasons.

The machine generates heat and because of the large size of the blending vessel such solvents as n-pentane and di-ethyl ether have a too high explosive hazard. However 'Freon 113' chloroform and methanol can be used. It is important that all solvents used in this process

have low boiling points because of the necessity of avoiding high temperatures in soxhlet extraction and because of the need for rapid removal of the solvent by evaporation.

With a suitable solvent the grinding in the large Waring Blender is rapid (a few minutes) and the vessel is easily cleaned. So far of the available solvents tried methanol is the most suitable because we have found 'Freon 113' and chloroform to be lacking in extractive ability for the time intervals available in the routine examination of large numbers of plants. Methanol however has the very distinct disadvantage that in addition to the pharmacologically active substances being sought it extracts all sorts of thoroughly undesirable substances. Such materials as fats, gums, rubbers and a variety of plastic like materials interfere in a decisive way with the preparation of extracts suitable for intravenous injection into animals. Experience now indicates that soluble aqueous extracts of the dried methanol fraction are well tolerated and that this is in general terms the most suitable fraction to use in the search for substances which act on the central nervous system.

If whole methanol fractions are to be used it seems best to suspend them in a suitable aqueous vehicle and to administer them intraperitoneally or orally.

2. Screening

(a) Preparation of extracts for injection.

Since the beginning of our work for the Army Chemical Center we have had great difficulty in developing methods for the detection of substances in plants having actions on the central nervous system. This we have felt is chiefly due to the animals being overwhelmed in other ways by large amounts of extraneous material knocking out the heart or circulation or by some massive toxicity obscuring the more sensitive criteria which we were seeking.

A partial solution to this problem now seems to have been achieved and further development along these lines is being pursued.

All the plant specimens referred to in these assays were ground and extracted for three hours in a soxhlet apparatus with diethyl ether. These extracts contain fats, waxes, gums, rubbers, resins and a number of plastic like materials which render the extracts more or less unsuitable for administration except perhaps by the oral route. We have however had some success in making preparations suitable for intravenous injection by further extracting these gummy preparations with water as follows:-

Each gummy extract is homogenised with a teflon homogeniser with a small quantity (1-2 mls) of water and the result centrifuged. The aqueous phase is then transferred to another tube and the process repeated twice. The yield of aqueous extract is about 4-6 mls. So far this is the only method which we have been able to develop that can be applied to the problem of finding substances in plants which act on the C.N.S.

(b) Preparation of extracts for submission to the Army Chemical Center.

With the equipment available the above method gives us barely enough material for routine testing in mice but has never given us enough for submission to an outside agency. In order to prepare such extracts each plant showing detectable activity has to be reinvestigated and methodology developed to increase yield or to increase the scale of operations to give adequate material for submission.

If in the first instance we had enlarged our capacity to provide material for screening and for submission of extracts we could not achieve the screening of several hundred plants each year.

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(b) Screening Procedures and Results

I. Anticholinesterase-like activity.

The detection of anticholinesterase activity in plants is of interest because such agents might disable either centrally or peripherally. Moreover such substances might have some ability to antagonise hallucinogens of the Ditran or atropine related type. The appearance of such activity in two plants Table A was therefore of relevance to the program.

Anticholinesterase-like activity.

Table A

Plant No.	Total original weight of plant available	Remarks		
		Family	Genus	Species
5397	150 grams	Rubiaceae	Psychotria	Tenicaulis
5633	3.4 Kg			

Plant #5397

This plant is referred by the natives in Peru as "Sacha Chilja" and it is a small plant and difficult to find. Moreover attempts to get further active supplies have been rendered difficult by the fact that the plant grows a blue fungus readily throughout its structure and the activity is lost. However we have a request in to our representative in Peru for a larger supply.

Plant # 5633

So far this plant has not been identified. It has a latex but does not seem to contain any alkaloids. Its pharmacological activity was originally detected in extracts obtained with diethyl ether. Subsequent further extraction with methanol gave much more active material and there is now some evidence that there is more than one pharmacologically active substance present. Work on the identification of the plant is proceeding together with further work on purification and examination of the active principles. An order for a large supply has been placed in Peru.

The anticholinesterase-like activity of these plants first became evident in mice. Eserine like convulsions and post-mortem movements of voluntary muscle for about ten minutes after death occurred. The latter phenomenon was new to us and we were able to reproduce it by the administration of eserine. In addition, typical symptoms of hyperactivity of the parasympathetic system in the mouse were seen.

In the cat potentiation and prolongation of acetylcholine action added to the idea that acetylcholinesterase was being blocked. Some progress on the isolation of this substance has been made.

II. Ryanodine Like Activity

Table B.

Plant No.	Weight of plant collected	Botanical Identification		
		Family	Genus	Species
5540	2.0	Rubiaceae	Psychotria	
5564	0.6 Kg	Flacourtiaceae	Ryania	Spruceana

The Ryanodine like activity of 5564 seems to be identical with ryanodine itself and since the plant is one of the Rynania its activity is probably due to ryanodine or something closely related to it.

When first examined 5540 had convulsive activity followed by death in rigidity that passed over rapidly into rigor mortis. Careful examination revealed considerable differences from ryanodine but when we came to investigate its pharmacological activity in detail later we found that the crude plant had lost its potency. Further supplies have been ordered from our representative in Peru and a special effort to preserve its activity when it arrives will be made.

III. Tryptamine potentiation (Amine oxidase inhibition). Tables C and D.

For a number of reasons chief of which was the availability of a useful test method, it was decided to see if any extracts showed ability to inhibit amine oxidase. The hallucinogenic alkaloid harmine does this and is readily detected by the test. Moreover the fact that several plants have shown this potentiality indicates the value of such studies.

Method

Intravenous tryptamine 55 mg/kg produces a well marked readily studied effect in mice which lasts for 90 ± 5 seconds. Hypotonia, body tremor bilateral placing type clonic movements of fore-paws, hunching of the back, lateral head movement, backward locomotion, straub tail and marked bradypnea and dyspnea occur. It is known that mono-amine oxidase inhibitors can potentiate the tryptamine response 100 fold. While phenothia-

zines and LSD 25 can abolish the response.

Mice that have received the extract during the toxicity experiments are given the tryptamine half an hour later and the duration of the resultant tremor analysed.

Table C

Plant No.	Weight Collected Kg.	Botanical Identification		
		Family	Genus	Species
5336	5.5	Dilleniaceae		
5373	1.6	Dilleniaceae		
5507	0.8	Combretaceae		
5550	1.3	Menispermaceae		
5600	6.8	Convolvulaceae	Prevostea	Sericea

Table D

Plant No.	Weight Collected	Botanical Identification		
		Family	Genus	Species
5340	2.4	Sapindaceae		
5478	0.19	Rubiaceae	Cephalis?	
5604	0.4	Menispermaceae	Abuta	Barbata

In both 5536 and 5507 administration of tryptamine after the plant extract caused death. Of the plants in Table C No. 5536 was the most potent and showed a 7 times increase in the duration of tryptamine tremors. Selected members of this group will be studied in greater detail later.

As mentioned previously chlorpromazine and LSD 25 abolish the tryptamine response in mice. In this connection the plants listed in Table D showed some reduction in duration of tryptamine effect. The possible significance of this requires further investigation.

IV. CNS Stimulation.

The plants in this group, Table E were classified as CNS Stimulants if they exhibited running, jumping, convulsions or generalised increased reactivity or any form of pronounced excitement. The fact that several of these plants may also at some phase of their actions produce a depression makes the classification somewhat arbitrary.

Table E

Plant No.	Weight Collected Kg	Botanical Identification		
		Family	Genus	Species
5313	0.5	Urticaceae	Phenax	Augustifolius
5389	0.5	Anonaceae		
5413	3.0	Rubiaceae	Joosia sp.	
5416	1.0	Anonaceae		
5435	0.6	Dilliniaceae		
5485	0.8	Menispermaceae		
5502	0.125	Aristolochiaceae	Klugii	
5533	0.9	Sapindaceae		
5536	1.1	Passifloriaceae	same as 5525?	
5544	4.2	Polygonaceae	Cocouloba	
5548	1.0	Bignonaceae		
5550	1.3	Menispermaceae		
5564	0.6	Anonaceae		
5567	9.0	Dilliniaceae		
5594	0.5	Malpigiaceae		
5609	0.8	Flocourtiaceae	Xylosma	Salzmanii
5616	3.6	Sapindaceae		

5. C.N.S. Depressants.

The plants in this group, Table F were classified as CNS depressants if this appeared to be their predominant effect. Paralysis except neuromuscular, loss of righting reflexes, decreased activity, and certain forms of disorientation were the usual criteria. It must be emphasised however that irritant material can cause marked reduction in activity and that mice showing depression at low doses may die by convulsions at higher doses.

Table F

Plant No.	Weight Collected	Botanical Identification		
		Family	Genus	Species
5307	0.3	Solanaceae		
5432	0.3	Menisperm	Odontocarya	Floribunda
5526	0.7	Apocynaceae	Tabernaemontana	sp.
5562	0.8	Flacourtiaceae	Carpotroche	?
5573	0.7	Passiflora		
5595	5.7	Rubiaceae	Warscewiczia	Coccinea
5604	0.4	Menisperm	Abuta	Barbata
5596	0.5	Menisperm	Abuta	