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About the Institute

The Hunt Institute for Botanical Documentation, a research division of Carnegie Mellon University, specializes in the history of botany and all aspects of plant science and serves the international scientific community through research and documentation. To this end, the Institute acquires and maintains authoritative collections of books, plant images, manuscripts, portraits and data files, and provides publications and other modes of information service. The Institute meets the reference needs of botanists, biologists, historians, conservationists, librarians, bibliographers and the public at large, especially those concerned with any aspect of the North American flora.

Hunt Institute was dedicated in 1961 as the Rachel McMasters Miller Hunt Botanical Library, an international center for bibliographical research and service in the interests of botany and horticulture, as well as a center for the study of all aspects of the history of the plant sciences. By 1971 the Library's activities had so diversified that the name was changed to Hunt Institute for Botanical Documentation. Growth in collections and research projects led to the establishment of four programmatic departments: Archives, Art, Bibliography and the Library.

June 29, 1987

Sr. Pablo Sanchez Vindas
Museo Nacional de Costa Rica
Apdo 749=1000
San Jose
Costa Rica

Dear Sr. Sanchez:

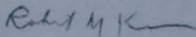
Please forgive the delay in writing to you as I lost your card and only now have recovered it.

I want to thank you for your help while I was in Costa Rica. I very much appreciate the time that you spent in helping us. I sent the labels and plants to Luis. I hope that you have received them.

I hope to visit Costa Rica next year if I can get the money.

With best wishes,

Sincerely,



Robert M. King

Department of Botany

SJD N° 325



UNIVERSIDAD NACIONAL DE ASUNCIÓN
FACULTAD DE CIENCIAS QUÍMICAS

Asunción, 4, September 1984

Mr.

Robert M. King
Smithsonian Institution
Washington D C 20560
U. S. A.

Digitized by Hunt Institute for Botanical Documentation

Dear Mr. King!

I am a member of the staff of the Faculty of Chemical Sciences of the National University of Asunción, and currently working with Prof. Bohlmann on the chemistry of Paraguayan Compositae.

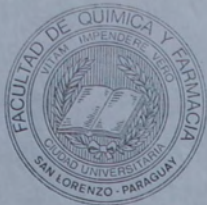
I am writing you this letter to ask you for the special favor of kindly identifying for me the accompanying voucher specimens.

Hoping to be of any help to you in the future and with gratitude,

I remain,

sincerely yours

Guillermo Schmeda Hirschmann
Facultad de Ciencias Químicas
Casilla 1055 Asunción
Paraguay



CINCUNETENARIO DE LA DEFENSA DEL CHACO

Universidad Nacional de Asunción
Facultad de Química y Farmacia

S JD 442/84

Asunción, December 9 th. 1984

Prof.
D r. Robert M. King
Smithsonian Institution
Washington DC 20560

U. S. A.

Dear Mr. King!

Digitized by Hunt Institute for Botanical Documentation

The 4th of September 1984, I sent you some voucher specimens
hoping you would kindly identify them for me.

Would you kindly let me know if you have received the material

Looking forward to an answer,

I remain

sincerely yours

Dr. Guillermo Schmeda Hirschmann
Facultad de Ciencias Químicas
Casilla 1055 Asunción-Paraguay

357-2546

December 17, 1984

Dr. G. Schmeda-Hirschmann
Facultad de Ciencias Químicas
Casilla 1055
Asuncion, Paraguay

Thank you for the specimens that you collected for chemical study by you and Dr. Bohlmann. A list of name is inclosed for you. I have also sent a copy of the list to Dr. Bohlmann in Berlin. I hope that you will be able to make more collections of speciemns for chemical study. I would ask that you look especially for members of the tribe Eupatorieae. Also, if it is possible, I would request that you collect living seed of the Eupatorieae or for that matter any Compositae. I am very intered in growing them for chromosome studies.

Thank you very much,

Sincerely,

Robert M. King
Department of Botany

List of names of Compositae

- E. Bordas 3172 *Stevia aristata* D. Don
- 1299 *Pterocaulon alopecuroides* (Lam.) DC
- 3169 *Disynaphia multicrenulata* (Sch. Bip. ex Baker) K. & R
- 3093 *Hypochoeris brasiliensis* (Less.) Benth. & Hook. ex Griseb.
- 3171 *Baccharis punctulata* DC
- 3070 *Pterocaulon polystachyum* DC
- 3117 *Campuloclinium macrocephalum* (Less.) DC
- 3109 *Verbesina subcordata* DC
- 1292 *Xanthium strumarium* L.
- 1297 *Achyrocline alata* (HBK) DC
- 1356 *Aspilia silphioides* (H & A) Benth.
- 1783 *Gamochaeta pensylvanica* (Willd.) Cabrera
- 1307 *Vernonia chamaedrys* Less.
- 1782 *Calea clematidea* Griseb.
- 3576 *Emilia fosbergii* Nicolson
- 3577 *Calea clematidea* Griseb.
- 1890 *Disynaphia multicrenulata* (Sch. Bip ex Baker) K. & R
- 3173 *Vernonia niederleinii* Hieron.
- 1836 *Vernonia niederleinii* Hieron.
- 3213 *Chromolaena laevigata* (Lam.) K & R
- 3104 *Calea uniflora* Less.
- 3105 *Bejaranoa balansae* (Hieron.) K. & R.
- 1887 *Austroeupatorium inulaefolium* (HBK) K. & R.
- 1760 *Tagetes erecta* L.
- 1528 *Chromolaena laevigata* (Lam.) K. & R.
- 1303 *Chromolaena odorata* (L.) K. & R.
- 1740 *Senecio grisebachii* Baker in Martius

Paraguay continued

- Bordas 1734 *Aspilia montevidensis* (Spreng.) O. Kuntze
 1531 *Calea clematidea* Baker
 1944 *Disynaphia multicrenulata* (Sch. Bip. ex Baker) K. & R.
 1938 *Achyrocline satureioides* DC
 1943 *Angelphytum arnottii* (Baker) H. Robinson
Chromolaena squarulosum H. & A # 1947
 1851 *Porophyllum ruderales* (Jacq.) Cass.
 1857 *Campuloclinium macrocephalum* (Less.) DC
 1856 *Achyrocline satureioides* (Lam.) DC.
 1833 *Acmella grisea* (Chod.) Jansen
 1834 *Conyza bonariensis* (L.) Cronq.
 1835 *Vernonia remotifolia* L.C. Rich.
 1889 *Trixis verbasciformis* Less.
 1616 *Praxelis clematidea* (Griseb.) K. & R.
 1306 *Baccharis punctulata* DC
 1525 *Mikania ypacarayensis* Holmes & McDaniel
 1620 *Bidens subalternans* DC
 1315 *Aster squamatus* (Spreng.) Hieron.
 1928 *Baccharis punctulata* DC
 1927 *Vernonia scorpioides* (Lam.) Pers.
 1601 *Aspilia montevidensis* (Spreng.) O. Kuntze
 1602 *Angelphytum arnottii* (Baker) H. Robinson
 1842 *Barrosoa candolleana* (H. & A.) K. & R.
 1839 *Grazielia serrata* (Spreng.) K. & R.
 1546 *Vernonia itapensis* Chod.

without collector indicated # 5001 *Galinsoga parviflora* Cav.

" " " # 5000 *Senecio grisebachii* Baker

July 10, 1985

Dr. G. Schmeda Hirschmann
Universidad Nacional de Asuncion
Facultad de Ciencias Químicas
Ciudad Universitaria
Casilla 1055, Asuncion, Paraguay

Dear Dr. Hirschmann:

Thanks for your recent letter. The specimens have not arrived yet, but I will get them named for you.

I would like to inquire of the possibility of my visiting Paraguay to make some collections of Compositae and general collections. I do not have very much money but if I could get to Asuncion, how much would it cost me? Would there be anyone at your institute that could help me in collecting? Is it very difficult to get around in the country side? I would like to spend about one month.

Would you please let me know what might be possible and how much it would cost?

Sincerely yours,

Robert M. King
Department of Botany

September 16, 1985

Dr. G. Schmeda Hirschmann
Universidad Nacional de Asuncion
Facultad de Ciencias Quimicas
Ciudad Universitaria
Casilla 1055
Asuncion, Paraguay

Dear Dr. Schmeda:

Enclosed is the list of names that you sent a long time ago.
I am sorry for the delay but I thought that the list was sent to
you earlier.

There is a plant that grows near Asuncion that I believe Prof.
Bohlmann would like to have biochemical material of. It is the
genus Gymnocoronis in the Compositae. If you can arrange to get it, would you
try to collect viable seeds for me? Thanks so much.

Sincerely,

Robert M. King
Department of Botany

October 24, 1985

Dr. Guillermo Schmeda Hirachmann
Universidad Nacional de Asuncion
Casilla 1055
Asuncion
Paraguay

Dear Dr. Schmeda:

Thanks so much for your letter which just arrived. I do not understand why it took so long to get here. Your specimens arrived much earlier. I expect that your letter was in our building all the time. We do have trouble with our mail. We could use the second set of specimens. Perhaps you could send another set of labels.

Regarding my trip to Paraguay, it will perhaps be possible for me to come during January or February. I have some teaching duties and this is really the only time that I can get away. I realize that this is not the best time and is very hot. I think that I can stand the heat if I wear a hat. Perhaps this is not a good time for you people.

The hotel that you suggest sounds good. The main thing is that it is clean. I do not have money to rent a car but would hope to go along with others that might give me a ride. I have already mentioned this to Dr. Bordas. She has suggested that I also might get some help from some Americans in the Peace Corps

Please let me know your reaction to the above.

Sincerely,

Robert M. King
Department of Botany

November 18, 1985

Dr. G. Schmeda H.
Universidad Nacional de Asuncion
Asuncion, Paraguay

Dear Dr. Schmeda:

Thanks for your letter of October 25, 1985. The specimen that you sent is *Senecio deferens* Griseb. We are working on the other specimens and will send you the names soon.

I will try to arrange my schedule so that I may visit Paraguay in March of 1986. It will be nice to meet you and go collecting.

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Robert M. King
Very sincerely,

Robert M. King

Department of Botany

December 30, 1985

Dr. Guillermo Schmeda H.
Universidad Nacional de Asuncion
Casilla 1055
Asuncion, Paraguay

Dear Dr. Schmeda:

Thanks for your letter of December 11th.

I am planning to visit you in March of next year. However, I still have a major problem. I am also planning to visit Bolivia first in February. The mail service is very poor there. So far I have not been able to complete arrangements with some people there.

Enclosed is the list of names of the plants that you sent to me. I will also send a copy to Professor Bohlmann.

I know that this letter will arrive late but I hope that you will have a Merry Christmas and a Happy New Year.

Sincerely,

Robert M. King
Department of Botany

March 10, 1986

Dr. G. Schmeda Hirschmann
Facultad de Química y Farmacia
Universidad Nacional de Asunción
Casilla 1055
Asunción, Paraguay

Dear Dr Hirschmann:

Please forgive the long delay in writing to you. The problem is that I had a very bad accident. I broke my leg in January(the femur). I am still on crutches for another month and then a cane for how long? So it looks like I will Not be coming to Paraguay for awhile. Sorry if I put out, but. I am still very eager to collect in Paraguay. Please tell Eugeneie of my problem. Thanks.

Sincerely,

Robert M. King
Department of Botany

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SJD 209/86

Cincuentenario de la Defensa del Chaco

Universidad Nacional de Asunción
FACULTAD DE CIENCIAS QUÍMICAS

Asunción, November 30th., 1986

Dr. R.M. King
Smithsonian Institution
Department of Botany
NHB # 166
Washington D.C.
U. S. A.

Dear Dr. King,

I hope you are fully recovered by now and collecting Compositae again. I am sending you some voucher specimens expecting you and your colleagues may help us to determine them.

As you will see, most specimens belongs to the family Myrtaceae. I want to explain you the reason: we are now working on paraguayan Myrtaceae and we need good determinations in order to do reliable phytochemical research. I would really appreciate if you would ask your specialist for those very important determinations. On September 19., I sent to Dr. Robinson a package containing plant specimens. Would you please ask him about them? Names of the Myrtaceae are necessary for publishing our preliminary results.

Eugenia and I are looking forward for your visit to Paraguay, whenever your health and spirit permit. Hoping to hear from you soon, I remain very truly yours. I also wish you and Dr. Robinson a merry Christmas and a happy New Year.

Cordially yours

G. Schmida Hirschmann

Casilla 1055-Asunción



Universidad Nacional de Asunción
FACULTAD DE CIENCIAS QUÍMICAS

Voucher Specimens

Leg.: G. Schmeda H.

- 763 Hierba 40-60 cm. A orillas de poza de agua. Dto. Pte. Hayes.
Km 30 de la ruta Puerto Militar-Concepción a partir de Pozo
Colorado. 10.09.86
- 764 Hierba 30-40 cm. Flores crema. Orillas del camino. Río Aguará
Guazú, Dto. Pte. Hayes. 10.09.86
- 765 Hierba 20-30 cm; inflorescencias blancas. A orillas del camino
Río Aguará Guazú, Dto. Pte. Hayes. 10.09.86.
- 766 Arbol 4 m; flores de pétalos blancos; frutos rojos. "Nangapiry"
Campus Universitario, San Lorenzo, Dto. Central. 03.10.86
- 767 Arbol 4 m; Campus Universitario; San Lorenzo. Flores de pétalos
blancos; frutos rojos. "Nangapiry". 03.10.86
- 768 Arbusto ca. 60 cm; frutos verde - rojos - negros. La Colmena.
Estancia Bertoni, Dto. Paraguari. 08.10.86
- 769 Sufruticosa? frutos inmaduros. La Colmena, Estancia Bertoni,
dto. Paraguari 08.10.86
- 770 Arbusto/sufrútice 1.5 - 2 m. En campos quemados. La Colmena.
Estancia Bertoni. 08.10.86. "Juí monehá".
- 771 Sufrutice? ca. 40-60 cm. Frutos rojos. "Nangapiry mí"
15.10.86. Emboscada, Compañía Itá Trompo, Dto Cordillera.
- 772 Arbusto 1.5-2 m. Frutos rojos. 15.10.86. Emboscada, Compañía
Itá Trompo, Dto. Cordillera.
- 773 Hierba, suelo modificado. Emboscada, Compañías Itá Trompo
15.10.86
- 774 Hierba 50-100 cm. Campus Universitario, San Lorenzo, Dto.
Central. Solidago chilensis Meyen



Universidad Nacional de Asunción
FACULTAD DE CIENCIAS QUÍMICAS

- 775 Arbol 5-6 m. Flores de pétalos blancos, frutos rojos. Asunción.
"Ñangapiry" 02.11.86.
- 776 Arbol 2-3 m; frutos maduros violeta-negros, en el tronco.
"Yvapuru". Emboscada, Compañía Itá Trompo, Dto. Cordillera.
14.10.86
- 777 Arbol ca. 5 m; frutos rojizos. Flores de pétalos crema. Emboscada, Compañía Itá Trompo. 03.11.86
- 778 Arbol ca. 3 m; flores de pétalos crema. A orillas de camino.
Emboscada, Compañía Itá Trompo. 03.11.86 "Guayaba"; "Arasá"
- 779 Arbol ca. 6 m; selva degradada, Patiño, frutos amarillentos.
06.11.86. "Yvapai". Dto. Central.
- 781 Arbol ca. 3-4 m; en selva degradada. Frutos violeta-negros.
"Yvapuru". Patiño, Dto. Central 05.11.86
- 783 Sufrútice ca. 50 cm. "Ñangapiry mí". Suelo modificado a orillas de selva degradada. Patiño, Dto. Central. 06.11.86
- 784 Arbol 1.5 m; orillas de selva degradada. Patiño, Dto. Central.
Frutos rojos. "Ñangapiry". 06.11.86
- 785 Arbol 2 m; orillas del camino. "Arasá". Patiño, Dto. Central
06.11.86
- 786 Arbol ca. 2 m; selva degradada, orillas de arroyo. Patiño, Dto. Central. Frutos rojizos. "Yvaporoity". 06.11.86
- 787 Arbol 4-6 m; frutos negro violáceos. Selva degradada. Suelo arenoso. Patiño, Dto. Central. 06.11.86
- 789 Sufruticosa; ca. 50 cm, suelo arenoso ex-selva. La Colmena, Estancia Bertoni, Dto. Paraguari. 11.11.86

- 790 Arbol 4-6 m; en "isla" de selva; Estancia Bertoni, La Colmena,
Dto. Paraguari. Frutos rojos "Nangapiry". 11.11.86
- 791 Arbol ca. 4 m. En "isla" de selva. Estancia Bertoni, La Colmena,
Dto. Paraguari. "Caá ovetí" 11.11.86
- 792 Arbol ca. 3 m. Campo ex-selva. Estancia Bertoni, La Colmena.
11.11.86 "Arasá pé yvaté".
- 793 Sufrútice? 30-50 cm. Frutos rojos, comestibles. Campos ex-selva
Estancia Bertoni, La Colmena, Dto. Paraguari 11.11.86
"Nangapiry ñú".
- 794 Hierba, inflorescencias azulinas, suelo modificado. Estancia
Bertoni, La Colmena, Dto. Paraguari 11.11.86
Leg.: G. Schmeda Hirschmann

December 15, 1986

Dr. G. Schmeda Hirschmann
Universidad Nacional de Asuncion
Facultad de Ciencias Químicas
Casilla 1055
Asuncion, Paraguay

Dear Dr. Schmeda,

Thanks for your letter. It was good to hear from you again.

Your specimens have arrived. I have asked that they be identified by one of our curators, and they have indicated that they will do their best. It seems that the Myrtaceae is rather difficult to identify.

I am still hoping to get to Paraguay some day. My leg continues to improve.

I wish you and Eugenia a very Merry Christmas and a Happy New Year.

Sincerely,

Robert M. King
Department of Botany

P. S. Would you be interested in working on the family Acanthaceae?
We have a curator that would like to cooperate with you on this.

RMK

BOTANISCHE STAATSSAMMLUNG
MÜNCHEN

Direktor: Prof. Dr. H. Merxmüller

Menzinger Strasse 67
Telefon 089/1792-1 - 252-

D-8000 München 19, 4. 09. 1981

Tgb, Nr.: 1471/q

Dr. R. M. King
Smithsonian Institution
U. S. National Museum
Department of Botany
WASHINGTON D. C. 20560
U. S. A.

Dear Dr. King,

Professor Merxmüller is still on a convalescent leave: 3 months he has stayed in the hospital because of various health troubles, then he had to cure in a "spa". But now it is to be hoped that he is on the way to recover from all the medical treatment!

When I informed him that among other exchange material ^{*)} 25 Compositae from your Brazilian collection have arrived, he asked me to thank you very cordially, not only for this new interesting set of duplicates, but also for the excellent collection of plant photographs he received at an earlier date, and which he is anxious are left without his thanks up to now.

With kind regards,
Yours sincerely,

*) from
"Smithsonian"

Annelis Schreiber

Dr. A. Schreiber
for the Director

357-2545

June 28, 1982

Mr. Keith Seager
RD # 2
Box 111C
Cape May, NJ 08204

Dear Mr. Seager:

I wanted to write and thank you for your help in locating Sclerolepis on Sunday. I followed your directions and found many of the plants for my study.

Very sincerely,

Digitized by Hunt Institute for Botanical Documentation

Robert M. King
Department of Botany

RMK:cap

357-2545

September 23, 1982

Dr. Frederick Seaman
New York Botanical Garden
Bronx, N. Y. 10458

Dear Dr. Seaman:

Would please send me a copy of your Sesquiterpene Lactones ---
that recently appeared in the Botanical Review. Thank you.

Sincerely,

Robert M. King
Department of Botany

December 22, 1986

Dr. Tatiana Sendulsky
Herbario do Estado "Maria Encyda P. K. Fidalgo"
Instituto de Botanica
Caixa Postal 4005, 01000
Sao Paulo, Sao Paulo
Brasil

Dear Tatiana,

I hope that you had a good trip home. It was nice having you here again. Tom is back to work and is looking better.

I have put off my collecting trip to Brasil until 1988. It seems that it will take too long to get the permit. Instead I am going to Bolivia for the month of February. I will, however, be coming to Rio and Sao Paulo on my way. I will be in Sao Paulo on the 28 January to 31 January. I would like to visit your herbarium. With that in mind would it be possible for me to stay in the room for visitors at your Institute?

I wonder also if there is anyway that I could do some collecting while I am there. Of course, I would be willing to pay for a car.

Please let me know what is possible.

Sincerely yours,


Robert M. King

Department of Botany

August 20, 1987

Dr. P. S. Short
National Herbarium of Victoria
Royal Botanic Gardens
Birdwood Avenue
South Yarra
Victoria 3141
Australia

Dear Dr. Short:

I would like to inquire if you have published the names of the two new genera of Compositae that I collected in Western Australia last year. They are my numbers 9558 and 9572. Vouchers of both numbers should be deposited at Perth. In addition, I would be very eager to send the USNH material for your study. I need the names so that we can publish the chemical data.

I hope that your work is going well. I enjoyed meeting you.

Digitized by Hunt Institute for Botanical Documentation

Sincerely,

Robert M. King
Robert M. King
Department of Botany

November 19, 1987

Dr. P. S. Short
Royal Botanic Gardens and National Herbarium
Birdwood Avenue
South Yarra
Victoria 3141
AUSTRALIA

Dear Philip,

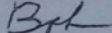
Thanks so much for your letter. I am sorry to take so long in answering you. I hope that you had a good vacation.

I am having the sheets of the new genera sent to you on loan. I decided to include some of the other genera that you are studying. I hope that you do not mind. Perhaps when you have decided on the names of the new genera you could let me know. We could then publish the chemical results. It usually takes a year to publish in Phytochemistry. Let me know what you think.

My new book on the Eupatorieae is out and I am including some information about.

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Most sincerely,



Robert M. King
Department of Botany

P.S. I am planning to visit Australia next year. I plan to go to Queensland, New South Wales and Victoria. I hope to see you again.

October 5, 1988

Dr. P. S. Short
Royal Botanic Gardens and National Herbarium
Birdwood Avenue
South Yarra, Victoria 3141
AUSTRALIA

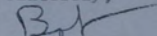
Dear Philip,

Thanks so much for your letter and the names of your new genera. I wonder if you could let us know the relationships of the new genera or better yet would you be willing to co-author the paper on their chemistry? I could ask Prof. Bohlmann to invite you to work with us. I do not believe that the chemistry has been worked up on these genera yet so I do'nt know if there is anything good or not.

Right now, I am planning to come to Australia in late December and do some collecting for about one month. I plan to start my trip in Victoria and perhaps collect in South Australia and New South Wales. Would be willing to name my collections? I would also need some help in shipping material to Bohlmann in Berlin. Would you please write me a letter inviting me, I need this for the visa. I expect to bring my assistant, Dr. Eric Hennz along to help me.

Please forgive the long delay in answering your letter as I was away on a trip. I hope that your work is going well.

Sincerely,


Robert M. King

Department of Botany

April 10, 1989

Dr. Phillip Short
Royal Botanic Gardens and National Herbarium
Birdwood Avenue
South Yarra
Victoria 3141, Australia

Dear Phillip,

Enclosed are two reprints that might be of interest to you.

Please forgive the delay in writing to you but I have just recently returned from a short trip to Europe.

The labels are keyboarded in the computer and will be printed as soon as we have the names from you. Incidentally, the money for shipping the specimens should have been sent more than two weeks ago. Let me know if it has not arrived.

I hope that my collections have been interesting to you.

With kind regards,

Sincerely,

Robert M. King

Department of Botany

P. S. Are you going to the Kew Conference?

May 8, 1989

Dr. Philip S. Short
National Herbarium of Victoria
Birdwood Avenue
South Yarra
Victoria 3141
Australia

Dear Philip,

Many thanks for the list of names for my collections. I very much appreciate your working on them. Thanks also for your reprints. We will make good use of them. The labels have been printed and typed and I will try to send them today. I have also sent you ca. 10 more sheets that I collected later in my trip. There is also a duplicate lichen for your herbarium.

I have just learned that one label still needs to be typed so I will send them to you later this week.

Sincerely,

Robert M. King

Department of Botany

Digitized by Hunt Institute for Botanical Documentation

June 20, 1989

Dr. P. S. Short
National Herbarium of Victoria
Birdwood Avenue
South Yarra
Victoria 3141
Australia

Dear Philip,

Thanks so much for the last list of names. With regard to the duplicates, we will send a set to K, MO, G, and GH. I expect that my collections are of rather common species and are well represented in Australian herbaria.

Enclosed please find copies of the picture that I had my assistant take of you. I hope that you like them as I am sending other copies to our Hunt Library in Pittsburgh. They will no doubt contact you.

Enclosed also 2 reprints on chromosome numbers. We are now planning a paper on chromosome number of the Senecio tribe. I wonder if you have any unpublished numbers of Senecio that you might want include in our paper. Of course, you would be among the authorship. Let me know

Please include the stop # 166 in my address. There are over 1200 people working in this building alone!

Please thank everyone again for me.

Sincerely,

RM
Robert M. King
Department of Botany

October 25, 1989

Dr. P. S. Short
National Herbarium of Victoria
Birdwood Avenue
South Yarra
Victoria 3141
Australia

Dear Phillip,

Just a short note to ask if you would name some collections that I expect to make of *Compositae* in Queensland and New South Wales in December of this year. The collections would be vouchers for our chloroplast DNA studies.

Hope that all is well with you. Please give my best regards to Helen and Jim.

Sincerely,



Robert M. King

Department of Botany

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Department of
Conservation
Forests & Lands

Your Ref:
In reply
please quote:
Contact:

Royal Botanic Gardens
and National Herbarium

Birdwood Avenue
South Yarra Victoria 3141
Telephone (03) 650 9424
Facsimile (03) 650 5917

2 November 1989

File 5.2.16

Dr R. M. King,
National Museum of Natural History,
Smithsonian Institution,
Washington, D.C. 20560

Dear Bob,

I do not wish to spend time naming your collections of Compositae from Queensland and New South Wales. The fact is staff at both of the respective State Herbaria undoubtedly have a better knowledge of their local flora than I do. In any case I think it only courteous, and possibly obligatory with some permits, to present both BRI and NSW with what I'm sure will be excellent duplicates.

Yours sincerely,

P. S. Short

357-2546

Dec 6, 1982

Dr D.N. Smith
C/O Dr Hernandez de Macedo
Museo de Historia Natural
Casilla 11434
Lima 14, Peru

Dear Dr. Smith:

Thank you for your recent. I will
pick up the jeep at the museum as you
have suggested.

Thank you for all the other information
regarding permits etc. I hope to meet
you in Lima.

Sincerely
Robert M. King

March 2, 1983

D.N. Smith
C/O Dr. Hermando de Macedo
Museo de Historia Natural
Casilla 11434
Lima 14, Peru

Dear David,

I have a big request to make of you.
It seems that all my plant collections
did not get packed. Probably about
one bundle

the last #s that I collected
The number are 7286-7306

dryed these for me and should still
be there some place. Enclosed is
a mailing frank. If you wrap them
and take them to the Agricultural
Attaché at the U.S. Embassy they
will mail them for you. I hope
that you find them. I am also
writing Ramon. Thanks a lot

Sincerely
Bot King

P.S.
I have regretted them
the regrets

357-2545

February 23, 1981

Dr. Jose Manoel Soares Nunes
Jardim Botânico do Rio de Janeiro
Secao de Botanica Sistemática
Rua Pacheco Leao 915
22.460 Rio de Janeiro
R. J. Brasil

Dear Dr. Soares:

Thanks so much for sending your paper on Compositae. I would like very much to see your types and have written to Dr. Franklin. Your help is appreciated.

Sincerely,

Digitized by Hunt Institute for Botanical Documentation

Robert M. King
Department of Botany



University of Illinois at Chicago

HEALTH SCIENCES CENTER

COLLEGE OF PHARMACY
Program for Collaborative Research
in the Pharmaceutical Sciences
World Health Organization,
Collaborating Centre for Traditional Medicine

833 South Wood Street
Chicago, Illinois 60612
Mailing Address: P.O. Box 6998
Chicago, Illinois 60680
(312) 996-7253

May 2, 1984

Ref. No. SX-58

Dr. Robert M. King
Botany Department NHB 166
Natural Museum of Natural History
Smithsonian Institution
Washington, D.C. 20560

Dear Bob:

It was a pleasure to be able to meet you again and talk with you about Stevia and my grant proposal to the NSF. Fortunately, later on that day (April 25) I also talked with Harold Robinson, who candidly expressed his points of view to me about the Stevia taxonomy, which I appreciated.

Harold asked me about the carcinogenicity of stevioside and rebaudioside, since such information was included in your big review which is forthcoming. I then promised to send you/him a copy of the work done by our group which showed the lack of any mutagenicity of these compounds. Please find it enclosed. As you will notice, however, steviol, the aglycone of stevioside and rebaudioside A, is mutagenic.

I would appreciate it if you send me the copy of the proposal which I will submit to NSF, with your annotations/critique as soon as possible, so that I could incorporate them into the original. The deadline for submission is sometime in June, but I want to send it in by mid May.

Please convey my regards to Harold Robinson.

With best personal regards.

Truly yours,

Doel Soejarto
PCRPS, College of Pharmacy
Univ of Illinois at Chicago
833 S. Wood St., Chicago
IL. 60612

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APPENDIX II

NOTICE OF RESEARCH PROJECT
SCIENCE INFORMATION EXCHANGESMITHSONIAN INSTITUTION
NATIONAL SCIENCE FOUNDATION
PROJECT SUMMARYPROJECT NO. (Do not use
this space)

NSF AWARD NO.

1. NAME OF INSTITUTION (INCLUDE BRANCH/CAMPUS & SCHOOL OR DIVISION)
PCRPS (PROGRAM FOR COLLABORATIVE RESEARCH IN THE PHARMACEUTICAL SCIENCES), COLLEGE OF PHARMACY, UNIVERSITY OF ILLINOIS AT CHICAGO
2. MAILING ADDRESS
PCRPS, 833 South Wood St., Chicago, Illinois 60612
3. PRINCIPAL INVESTIGATOR AND FIELD OF SCIENCE/SPECIALTY
Djaja Doel Soejarto
4. TITLE OF PROJECT
STUDIES OF SOUTH AMERICAN STEVIA (COMPOSITAE - EUPATORIEAE)
5. SUMMARY OF PROPOSED WORK (LIMIT TO 22 PICA OR 18 ELITE TYPEWRITTEN LINES)
The genus <i>Stevia</i> comprises ca. 220 species of herbs and shrubs distributed from southern U.S. to Argentina and Brazil, through the Andes of South America. The proposed research will study the taxonomy of the South American members of the genus, which includes approximately 140 species; a taxonomic revision of the North American members has recently been completed (Grashoff, 1972). Studies will include analysis of herbarium specimens and field work in Argentina, Brazil, Paraguay, Bolivia, Peru, Ecuador, Colombia, and Venezuela. Other studies on pollen morphology, cytology, phytochemical screenings aimed primarily at flavonoid and diterpene glycosides, will also be performed. These studies are expected to provide new data, which will be used to elucidate the taxonomy and evolution of the genus, and may contribute new insights in elucidating the taxonomy and phylogeny of the tribe Eupatorieae as a whole.

FOR NSF USE ONLY

DIVISION (OFFICE) AND DIRECTORATE	PROGRAM	
SECTION	PROPOSAL NO.	F.Y.

FOR OGC USE ONLY

START AND END DATES	AMOUNT GRANTED
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STUDIES OF SOUTH AMERICAN STEVIA
(COMPOSITAE - EUPATORIEAE)

1.0 INTRODUCTION

Stevia is a New World genus of the Compositae, comprising approximately 220 species, distributed from southern U.S. to Argentina and Brazil (Fig. 1). Members of the genus comprise herbs (annuals and perennials) and shrubs, found mostly at altitudes of 500-3000 m above sea level. Though they usually grow in semidry mountainous terrains, their habitats range from grasslands, scrub forest, forested mountain slopes, conifer forests and subalpine vegetation.

The Principal Investigator (Dr. D.D. Soejarto) became acquainted with this plant group when he served as a Co-Principal Investigator of a research project entitled "Studies to Identify, Isolate and Test Naturally Occurring Noncariogenic Sweeteners That May Be Used as Dietary Sucrose Substitutes", under contract No. NIH- NO1-DE-02425, from the National Institute of Dental Research (Principal Investigator: Dr. A.D. Kinghorn), for the period of June, 1980 - September, 1983. One of the objectives of this project was to screen all available species of *Stevia* for the sweet diterpene glycosides (primarily stevioside and rebaudioside A), which had been previously isolated from a Paraguayan species, *Stevia rebaudiana* Bertoni.

During the course of the previous investigation, Dr. Soejarto surveyed the taxonomy of *Stevia* and examined more than 1,000 herbarium specimens of this genus from its entire geographic range, representing about 130 species, in deposit at the Field Museum of Natural History. (See Appendix I, Exhibit 1.) He also searched the taxonomic and non-taxonomic literature, carried out field studies in Paraguay, Peru, Colombia and Mexico to perform organoleptic tests and to collect *Stevia* herbarium specimens, samples and seeds. Later, he identified hundreds of *Stevia* specimens collected by him and by others, from the entire range of the genus. Results of these studies have been published (Soejarto et al., 1982, 1983a-b; Kinghorn et al., in press).

The present project will attempt to elucidate the taxonomy and phylogeny of this plant group. Because of his involvement with the taxonomy of the genus during the past three years, the Principal Investigator, whose training is in systematic botany (Ph.D., Harvard University, 1969) and who has published 35 scientific papers, of which more than 10 in plant systematics, is uniquely qualified to perform this task. He also has an extensive field work experience in South America, where he has participated in and conducted botanical expeditions in Brazil, Paraguay, Bolivia, Peru, Ecuador and Colombia, and has accumulated 5,700 numbers of collection (representing more than 20,000 plant specimens and ca. 500 different phytochemical



Figure 1. Generalized distribution of Stevia.
According to Grashoff (1972), 79 species occur
in North America (north of the Isthmus of Panama).
According to currently available data, approx.
140 species occur in South America.

samples), including several new species and at least a new genus. He is fluent in Spanish, and reads and writes in Portuguese.

2.0 PROPOSED RESEARCH

It is proposed to carry out biosystematic studies of South American *Stevia*, which will include herbarium analysis, field work in South America, studies on cytology, pollen morphology, and flavonoid and diterpene glycoside chemistry. Data obtained from these studies will be used to elucidate the taxonomy of the South American species, and to reconstruct the origin and evolution of the genus as a whole.

3.0 SPECIFIC OBJECTIVES

- a. To study herbarium collections of all the species of *Stevia* from South America, and relevant material from Central and North America. (For the remainder of this proposal the region from the Isthmus of Panama northward will be referred to as North America.)
- b. To carry out field trips in Brazil, Paraguay, Argentina, Bolivia, Peru, Ecuador, Colombia, Venezuela and Mexico to study species of *Stevia* in their natural habitats, to determine their eco-geographic distribution, and to collect herbarium specimens, cytological material, leaf samples for chemical studies, and seeds for cultivation experiments.
- c. To perform chromosome counts on as many representative species of *Stevia* as possible, but primarily those from South America.
- d. To study the pollen morphology of as many representative species of *Stevia* as possible, primarily from South America, by the use of light and scanning electron microscopy.
- e. To study the flavonoid, sesquiterpene and diterpene glycoside chemistry of as many representative species of *Stevia* as possible, throughout its geographic range, and those of the taxonomically related genera.
- f. To produce keys of identification for the South American species.
- g. To propose a scheme for the classification of *Stevia* and to reconstruct the possible origin and evolution of the genus, based on data derived from studies in a-f.

4.0 ANTICIPATED SIGNIFICANCE OF THE PROJECT

Firstly, *Stevia* is one of five truly large genera with over 100 species each, out of 160 within the tribe Eupatorieae, none of which has been completely monographed in recent years

(Robinson & King, 1977). A comprehensive study of the South American species of this genus will complement our knowledge on the recently completed studies of the North American species (Grashoff, 1972).

Secondly, one member of *Stevia* (*S. rebaudiana* Bertonii) possesses non-caloric sweet compounds (300x sweeter than sucrose) which are widely used in Japan as a sugar substitute, and in other countries in a lesser scale, thus making it a commercially important species. Detailed knowledge on the systematics of the genus as a whole will greatly facilitate further studies in relation to agricultural and genetic improvements of this species.

Thirdly, the results of the proposed research will provide:

- a. An up-to-date inventory of all the species of *Stevia* from South America, which will form the basis for a future monographic treatment.
- b. Taxonomic keys for the identification of the South American specimens of *Stevia*.
- c. New information on the taxonomic value and distribution of secondary constituents (flavonoids, sesquiterpene lactones, diterpene glycosides) within *Stevia* and its related genera.
- d. A scheme of classification and phylogeny of *Stevia*.
- e. A distribution map on the occurrence of sweet diterpene glycosides within the genus, which may be useful from both chemotaxonomic and economic points of view.
- f. New data for understanding the systematics of the tribe Eupatorieae and the family Compositae as a whole.
- g. A substantial addition to existing herbarium collections of *Stevia*, particularly from South America.

5.0 RELATIONSHIP WITH OTHER CURRENT RESEARCH ON THE SYSTEMATICS OF THE COMPOSITAE - EUPATORIEAE

The specialist of the genus *Stevia* was the late Dr. Jerold Lee Grashoff, who completed the monographic revision of the North American portion of the genus (Grashoff, 1972). Sadly, he died before completing his work on the whole genus.

For South America, there has been no attempt to treat the genus throughout its entire geographic range during the past 50 years, except for several isolated studies (Herter, 1939; Koster, 1945, 1948; Cabrera, 1974, 1976; Cabrera & Vittet, 1963).

Even the current authorities of the tribe Eupatorieae, R.M. King and H. Robinson (1977) are not contemplating to monograph

the South American portion of this genus at the present time or in the near future (personal communication with R.M. King).

6.0 CURRENT STATUS AND PROBLEMS ON THE SYSTEMATICS OF *STEVIA*

6.1 Historical Review

The genus *Stevia* was established in 1797 by Cavanilles, based on specimens collected from cultivated plants at the Royal Gardens in Madrid (Robinson, 1930a). These plants were raised from seeds or stocks brought from Mexico.

Within the first 30 years of its history, the systematic study of the genus consisted mainly of the description of new species, by various authors, as they were discovered, primarily from Mexico.

In 1836, A.P. De Candolle revised the genus and recognized 68 species, mostly from North America. In 1852, Schultz-Bipontinus presented an account of the genus and recognized 22 species, also mostly from North America. In 1876, Baker produced an account of 28 species of *Stevia* from Brazil (which included Uruguayan species), and in 1897, Hieronymus provided an account of 45 species of the genus from Argentina and the neighboring countries (Uruguay, Brazil, Paraguay, Bolivia).

Between 1900 and 1932 the study of *Stevia* was the domain of B. L. Robinson, who described new species and attempted country-by-country revisions of the genus, by completing the treatments for Argentina (1930b), Paraguay (1930c), North America (1930d), Colombia (1931a), Venezuela (1931b), Ecuador (1931c), Bolivia (1932a) and Peru (1932b).

Until the early 1970s, Robinson's treatises were the only work available for the identification of *Stevia* specimens, except those from Brazil, for which Baker's somewhat outdated treatment is the only one available. The genus was typified in 1969 by King & Robinson, who selected *Stevia serrata* Cav. as the lecto-type.

In 1972, Grashoff completed the monographic treatment of the North American *Stevia*, in which 79 species, including many new to science were recognized. This was a thorough treatment, based on examination of more than 9,000 specimens and field works in Mexico.

6.2 Taxonomic Problems

A. Delimitation of Species

A survey of this genus indicated that at least 385 Latin binomials have been published (Soejarto et al., 1983). Recent estimates, however, place the number of species between 150 and 300 (King & Robinson, 1967; Grashoff, 1972; Robinson & King, 1977). This means that between 85 and 235 Latin binomials that have been published for *Stevia* are mere synonyms, and

indicates the lack of unifying criteria for species delimitation within the genus. This may be due to the fact that this plant group has never been studied throughout its entire geographic range.

Based on the taxonomic revisions of B. L. Robinson (1930b-c, 1931a-c, 1932a-b) and Baker (1876), 126 species of *Stevia* are registered for South America, excluding Chile. For the latter, five species have been described (Index Kewensis, vols. 1, 2, suppl. 1-16). Since at least 11 species of *Stevia* from South America new to science have been described between 1932 and 1975 (Index Kewensis, suppl. 10-16), an estimate of 140 species ~~of~~ ^{for the genus} *Stevia* from South America may be given.

In the light of our present knowledge on the biological species concept, it is obvious that B. L. Robinson's species definition should be re-examined and up-graded. Besides, during the past 50 years a significant addition to the South American material studied by Robinson has been made, which may shed some light on the criteria for the delimitation of species of *Stevia*.

For North America, Robinson (1932d) recognized 67 species of *Stevia* and between 1930 and 1971, a total of 8 new species were described. Grashoff (1972, 1974) described 13 new species and reduced 9 Latin binomials into synonymy, making the total number of species accepted to 79.

Figs. 2 and 3 present the known distribution of North and South American *Stevia*.

B. Delimitation of Subgeneric Divisions

Another taxonomic problem in *Stevia* involves the grouping of species into subgeneric divisions.

Robinson (1930b-c, 1931a-c, 1932a-b) recognized 3 subgeneric Sections, in a somewhat cryptic manner, for the South American *Stevia*, namely *Breviaristatae*, *Eustevia* and *Multiaristatae*. For North America (1930d), he recognized 2 subgeneric Series, *Podocephalae* and *Corymbosae*, the latter further divided into 2 Subseries, *Fruticosae* and *Herbaceae*.

On the basis of pappus type, inflorescence characteristics, and habit of the plant, Grashoff (1972) proposed 3 subgeneric Series for the North American *Stevia*, namely *Podocephalae* (16 species), *Corymbosae* (37 species) and *Fruticosae* (24 species). Provisionally, he assigned the South American species into 2 subgeneric Series, *Multiaristatae* and *Breviaristatae*.

Clearly, new evidence is badly needed for the recognition of a more natural and unified concept of the subgeneric divisions and their category within *Stevia*. Data to be produced by the proposed research are expected to contribute to the elucidation of this problem.



Figure 2. Concentration of species of Stevia in North America. Each dot represents one species for a particular area. For each country one species may be represented by more than one dot, depending on the occurrence of that species in the area. Number in front of slash = endemic species. Number behind slash = total number of species in country.

6.3 Phylogenetic Problems

On the basis of information available to him, Grashoff (1972) postulated two alternative hypotheses on the origin and evolution of *Stevia*:

- a. That the genus initially developed and evolved in central South America into Series *Multiaristatae* and *Breviaristatae* and, following evolutionary radiation, further developed in northwestern South America, and later Mexico.
- b. That the genus initially developed and evolved in northwestern South America and, following evolutionary radiation, further evolved into Series *Podocephalae* and *Fruticosae* in North America, and into Series *Multiaristatae* and *Breviaristatae* in South America.

The following evidence was presented as basis for the formulation of his hypotheses:

- a. *Multiaristate* and *Breviaristate* *Stevias* are both well represented in the central portion of South America.
- b. *Multiaristate*, *Breviaristate* and *Exaristate* *Stevias* are all represented in the northwestern part of South America.
- c. Series *Podocephalae* is almost equally well represented in North and South America, but scarce in central South America.
- d. Series *Corymbosae* are best represented in Mexico, but members occur and are scattered in the northwestern and central portions of South America.
- e. Series *Fruticosae* is restricted to North America, except for one species, which extends to northwestern South America.
- f. Apomictic reproduction occurs in members of the primarily North American Series *Podocephalae* and *Corymbosae*, but absent in the South American *Stevias*.

A careful review of the above list shows that some of the data presented are not supported by adequate evidence. The following is an analysis of the situation.

i. Morphology:

The most important criteria in the subgeneric grouping of species of *Stevia* are (1) pappus type, (2) inflorescence type and characteristics, and (3) habit of plant. Although Grashoff's analysis of these attributes was thorough for the North American species, his study of the South American counterpart was deficient. For example, based on Robinson's revisions (1931a-c), only two species of *Multiaristate* *Stevia* are found in the northwestern part of South America, viz. *S. andina* and *S. crenata* from Ecuador (Robinson,

1931c). Similarly, the number of Podocephalous and Corymbous Stevias for South America is not known with certainty, and the exact number and geographic range of both Multiaristate and Breviaristate species are also not precisely known.

As a result, comparison based on disparate knowledge between North and South America cannot give us a correct picture on the evolutionary trends between members in the two geographic regions. A complete over-all picture may be obtained only when members of both elements are equally well studied. Therefore, in order to postulate a more sound hypothesis, further study of the South American members of the genus is mandatory.

ii. Cytology:

Grashoff (1972) has established the basic chromosome number of the different subgeneric categories, as follows.

Subgeneric Category	Basic Number	Species	
		Total	Counted
North America:			
<i>Corymbosae</i>	X = 11, 12, 17	24	16
<i>Fruticosae</i>	X = 12	18	9
<i>Podocephalae</i>	X = 11	16	5
South America:			
<i>Breviaristatae</i>	X = 12	20 (?)**	1
<i>Eustevia*</i>	X = ?	40 (?)**	?
<i>Multiaristatae</i>	X = 11	60 (?)**	4

*Subgeneric division as proposed by Robinson (1930b-c, 1931a-c, 1932a-b). **Exact number of species not known.

As can be seen from the above figures, data for South America are far from adequate for making any postulation concerning the evolution of *Stevia*.

iii. Reproduction:

A major contribution made by Grashoff (1972) in the elucidation of the systematics of *Stevia* is his findings that a large number of the North American species have a facultative agamospermous (apomictic) mode of reproduction, compared to none for South America. Such observations provide

the basis for assuming that the North American Stevias are evolutionarily derived, and that the more primitive members of the genus should be sought in South America. Grashoff's findings were based on examination of the pollen characteristics of more than 50% of the North American species, supplemented by detailed study of the sporogenesis and gametogenesis of selected species. All apomictic species produce sterile and irregular pollen grains (in size, form,

type and symmetry), in contrast to the regular and uniform grains of normal, sexual species. As to sporogenesis, the major type of the apomictic development of the megaspore is described by Grashoff as pseudohomeotypic diplospory, and only rarely as semi-heterotypic diplospory.

With regard to the South American species, Grashoff examined the pollen morphology of 37 species (out of an estimated total of about 140). Although these data are quite adequate, further examination of the pollen morphology of the South American species to determine the occurrence of apomixis, if present, is desirable.

6.4 Pollen Morphology

The pollen morphology of *Stevia* was examined by King & Robinson (1967, 1968) by using light microscopy and was found to show two patterns: the South American species have uniform grain size and shape, whereas those of the North American species have more variable size, shape and symmetry, not only between, but also within species. Since no exine morphology was considered, it was concluded that pollen morphology does not seem to have any taxonomic significance, due to its uniformity in the South American species examined, or has only very limited value for the North American species. This finding agrees with that of Grashoff's, but he found that the highly variable pollen morphology of the North American species is due to apomixis.

Among a number of species of Brazilian Eupatorieae examined for their exine structure through light microscope and SEM (of fractured grains) by Mattozo dos Reis & Ferreira (1981) was a species of *Stevia*, *S. sellowii*, whose pollen is echinate and the exine classified as tectate with columellae, which are higher below spines, and cavate region between endexine and extexine. No other observations on *Stevia* pollen exine have been made.

In light of the statement by Skvarla et al. (1977) that the pollen morphology of the Eupatorieae is least studied compared to the others of the Compositae, and considering the poor data that are available for *Stevia*, it is proposed to examine the pollen morphology of as many representative species of this genus as possible, by using both light microscope and SEM, as an extension of Mattozo dos Reis & Ferreira's observations. Only after such a study is completed will we be able to conclude on the value of pollen morphology in the taxonomy of *Stevia*.

6.5 Chemotaxonomy

Though begun at the beginning of this century, phytochemical studies in *Stevia* until about fifteen years ago have been concentrated in the Paraguayan species *S. rebaudiana* Bertonii, mainly in the elucidation of its sweet-tasting *ent*-kaurene diterpene glycoside constituents, namely dulcoside A, rebaudiosides A-E, steviolbioside and stevioside (Kingham &

Soejarto, in press). So far only one other species of the genus, *S. phlebophylla* from Mexico, has been reported to contain sweet diterpene glycosides stevioside and rebaudioside A (Kighorn et al., in press). Non-sweet *ent*-kaurene glycosides, paniculoides I-V, have been reported to occur in *S. paniculata* Lag. (Yamasaki et al., 1977) and *S. ovata* Lag. (Kaneda et al., 1978).

Phytochemical studies on other species of *Stevia* were initiated in 1967, and to-date about 20 species of the genus have been investigated with the following classes of chemical compounds having been isolated from these species: bisabolane, germacrane, gualane, humulane, alpha-longipinane and pseudo-guaiane sesquiterpene derivatives, clerodane, kaurene and labdane diterpene derivatives, sterols, triterpenes, flavonoids, chromenes, euparine derivatives, and miscellaneous volatile constituents. Except for our own studies on the distribution of sweet diterpene glycosides in *Stevia* (Soejarto et al., 1982; Kinghorn et al., in press), phytochemical screening studies to determine the infrageneric distribution of either *ent*-kaurene glycosides or any other known *Stevia* constituents are so far lacking, thus the chemotaxonomic significance of all of the secondary metabolites so far isolated from plants of this genus is presently unknown (see also Harborne, 1977; Hegnauer, 1977; Herz, 1977).

An attempt in the use of chemical characters in solving a taxonomic problem was employed by Grashoff (1972), who investigated the flavonoids and volatile terpenes of selected species, in order to confirm the parentage of putative hybrids. This work consisted of comparison of the separation patterns of the flavonoid constituents on thin-layer chromatographic papers, and of the volatile terpenes by comparison of the separation patterns of the constituents as resolved on the gas-liquid chromatograms.

Since presently the flavonoid constituents in leaf or flower are considered among the most useful of chemical markers in the classification of angiosperm plants (cf. Harborne, 1974; Giannasi, 1975; Harborne, 1975; Crawford et al., 1980; Luteyn et al., 1980), the proposed research will study the flavonoid chemistry of as many species of *Stevia* as possible, throughout its entire range, in order to evaluate the significance of these compounds as taxonomic markers.

Additionally, phytochemical screening for sweet and non-sweet diterpene glycosides within the genus will also be carried out, the former as an extension of our previous work. If the complete distribution of these compounds in *Stevia* is known, their chemotaxonomic significance may become apparent.

Finally, in light of the review on the value of sesquiterpene lactones as taxonomic characters in the Compositae (Seaman, 1982), screening methods will be applied for this class of compounds, in an attempt to discern their chemotaxonomic significance in *Stevia*.

Because of our limited knowledge on other types of compounds so far identified from *Stevia*, and their lack of demonstrable value as chemotaxonomic markers, no studies will be performed on these compounds.

7.0 PROCEDURE

7.1 Systematic Study

Herbarium studies, field work and other stages of the taxonomic investigation (nomenclature, key construction), including synthesis of data in the postulation of hypothesis will be carried out by the Principal Investigator. Outside of field work, the major part of the taxonomic work will be carried out at the Field Museum of Natural History, where the Principal Investigator holds an appointment as a Research Associate.

7.2 Loan of Herbarium Specimens for Study

The Field Museum has a holding of approximately 1,700 *Stevia* specimens as the nucleus of the proposed research. In addition to this collection, loans of specimens will be requested from other botanical institutions. Important herbaria with large holdings of *Stevia* material will be identified, among which are the following:

U.S.A. : Harvard University herbaria (Arnold Arboretum and the Gray Herbarium), U.S. National Herbarium, the New York Botanical Garden, University of Michigan, and University of California at Berkeley.

Europe: The Kew Herbarium (England), Naturhistoriska Riksmuseum (Stockholm), Instituto "Antonio Jose Cavanilles" of Madrid (Spain), Museum National d'Histoire Naturelle (Paris), and Martin Luther Universitat (Halle, Germany).

South America: All national herbaria of countries where *Stevia* occurs.

All plant specimens will be handled with care and stored in metal cases, and will be annotated accordingly.

7.3 Procurement of Plant Samples

A small amount of leaf samples will be removed from representative specimens of each species for flavonoid study, and from selected specimens for sesquiterpene and diterpene glycoside screening. Similarly, pollen material will be collected, both for light microscopy and SEM examination. Permit will be secured from the loaning institutions for the removal of these samples for study.

In addition to the dried samples, fresh material will also be investigated. This will be collected from wild populations or harvested from cultivated plants (see 8.4 below).

7.4 Field Work

Three field trips during the period of the proposed project are scheduled, as follows:

First field trip: 6-10 weeks: Argentina, Bolivia, Peru, Ecuador, Colombia, Venezuela.

Second field trip: 6-10 weeks: Brazil, Paraguay, Argentina, Bolivia, Peru.

Third field trip: 6-10 weeks: Ecuador, Colombia, Venezuela, Mexico.

The dates and itinerary of each field work will be determined by study of herbarium specimens on hand and the literature, and will be planned in such a way, in order to maximize the results of the collection of flowering/fruitlet material. Photographs/xerox copies of specimens of selected species will be made, which will be taken to the field for on-the-spot identification. Permits to collect plants in each country to be visited will be requested far ahead of the anticipated field work date, in some cases over a year. This is the reason for scheduling the field work in Brazil for the second year, due to the long delay of securing permits to collect plants in this country.

Plant specimens and other plant materials, including liquid collections (cytological material), leaf samples and seeds, will be air-mailed or air-freighted to the U.S., whichever is more economical, but with assured arrival within a short time. In Chicago, an assistant will be in charge of opening and processing the plant materials, as they arrive.

For purposes of mobility, rented car/vehicle will be used for field work. Other modes of transportation will also be used in case rental vehicles are not available. From experience, rented vehicles are most productive in terms of terrain coverage in a relatively short period of time. If necessary, rented vehicles may be reserved ahead of time, if one uses vehicles from U.S. based rental agencies, such as the National Car Rental.

Attempts will be made to perform field work with local colleagues/botanists, to optimize success.

7.5 Chromosome Count

Chromosome counts will be made from flowering (bud) material, at diakinesis stage of meiosis. If necessary, mitotic figures obtained from root tips of germinating seeds will also be used.

Widely used fixative and stain for chromosome count (Carnoy's solution, acetocarmine stain) will be used in the squash techniques (Grashoff et al., 1972). Although the Principal Investigator has done chromosome counts in a different plant group (Soejarto, 1970), his experience will facilitate the

proposed work. Examination of squashed material will be made with a phase contrast microscope, and both photographs and camera lucida tracings will be made. A part-time graduate student (botany) under the supervision of the Principal Investigator will be employed in this part of research work.

7.6 Pollen Morphology

For the purpose of light microscopy, pollen will be acetolyzed according to the techniques described by Erdtman (1960, 1966). Pollen to be scanned (SEM) will also be acetolyzed, if necessary.

For the purpose of SEM, pollen for examination will be prepared and scanned by Mr. Ron Wibel, a SEM technician at the Biology Department of the University of Illinois at Chicago.

A part-time graduate student will be employed to carry out various phases of LM pollen studies, under the supervision of the Principal Investigator, and with the assistance of Dr. Sylvia Feuer, a palynologist at the Field Museum of Natural History. Interpretations of LM data and SEM photographs will be performed by the Principal Investigator, with the assistance of Dr. Sylvia Feuer.

7.7 Flavonoid Identification

The separation and identification of the flavonoids (aglycones and glycosides) will be performed according to established procedures (Seikel, 1962; Harborne, 1967; Mabry et al., 1970; Markham, 1975; Markham & Mabry, 1975; Daigle & Conkerton, 1982, 1983). Flavonoid-rich extract obtained by appropriate solvent fractionation will be subjected to preliminary identification by comparison with authentic standards of flavonoids known in the tribe Eupatorieae, using analytical TLC (single and two-dimensional) and HPLC. Structure work of individual flavonoids will be carried out when necessary after isolation (preparative TLC, low-pressure column chromatography) using standard spectroscopic methods (UV, IR, ^1H NMR, ^{13}C NMR, MS, GC/MS). Acid hydrolysis will be used to obtain the aglycone and sugar moieties of flavonoid glycosides. Members of our group have a limited experience in the isolation of flavonoids from *Stevia*, since chrysophenol D and luteolin-7-glucoside were obtained from *S. rebaudiana* leaves (Choi & Kinghorn, unpublished results).

7.8 Screening for Diterpene Glycosides

A phytochemical screening method designed to indicate the presence of sweet *ent*-kaurene glycosides from herbarium specimens has been developed by members of our group (Kinghorn et al., in press, see Appendix IV. This method involves a microextraction stage, followed by the comparative analysis by analytical TLC and HPLC of the sweet glycosides present in extracts compared with standard. Confirmation of identity involved acid hydrolysis of sweet *ent*-kaurene glycosides,

methylation, and detection of isosteviol methyl ester by GC/MS. In this manner, the most abundant sweet glycosides of a 60-year-old specimen of *S. rebaudiana* were detected, as well as trace amounts of stevioside in the Mexican species, *S. phlebophylla*. For the purpose of the present project, only species which have not been examined using the methodology developed for this purpose will be screened.

During our work on the screening of sweet *ent*-kaurene glycosides among the *Stevia* herbarium specimens examined, it was found that compounds of similar polarity were present in about half the extracts examined. It is possible that these compounds included non-sweet *ent*-kaurene glycosides similar to paniculoides I-V (Yamasaki et al., 1977; Kaneda et al., 1978). Therefore, we think it worthwhile to screen for the variously substituted *ent*-kaurene aglycones, or their rearrangement products, obtained either enzymatically or after acid hydrolysis. TLC and GC/MS (after silylation and methylation) could be used to detect these compounds. Authentic samples of the aglycones of paniculoides I-V, and similar compounds which have been found in the plant kingdom (kaur-16-en-18-oic acid and its 15-hydroxy, 12, 15-dihydroxy, 15-[(2-methyl-1-oxo-2-butenyl)oxy] derivatives) will be used for reference.

7.9 Preliminary Screening for Sesquiterpene Lactones

Sesquiterpene lactones can be detected by TLC by way of selective detecting (spraying) reagents (Drozd & Bloszyk, 1978). The chloroform extracts of the plant samples will be screened for this class of compounds, using analytical TLC. Further identification work will be achieved by reversed-phase HPLC (Strack et al., 1980; Marchand et al., 1983) and/or GC/MS (Perry et al., 1978; Pyysalo et al., 1980). Comparative analysis can then be made from the chromatograms.

8.0 LOCATION OF RESEARCH AND FACILITIES

8.1 PCRPS, University of Illinois at Chicago, 833 S. Wood St., Chicago, IL 60612.

The administration of the funds and all aspects of the phytochemical work for the proposed project will be performed at the PCRPS, located on the third floor of the College of Pharmacy Building. Facilities available at PCRPS for the conduct of the proposed research are outlined in APPENDIX III.

8.2 Botany Department, Field Museum of Natural History, Roosevelt Rd. at Lake Shore Dr., Chicago, IL 60605.

The major portion of the systematic work for the proposed project will be performed at the Botany Department of the Field Museum of Natural History.

Facilities available for the conduct of the proposed research are outlined in APPENDIX III.

8.3 Scanning Electron Microscopy Laboratory, 3422 SES, Biology Department, University of Illinois at Chicago.

SEM work for pollen morphology will be performed at the SEM Laboratory of the Biology Department by Mr. Ronald Wibel, a SEM technician. (See Appendix II, Exhibit 1.)

8.4 Horticulture and Pharmacognosy Field Station of the University of Illinois at Chicago, Downer's Grove, Illinois. Cultivation experiments will be carried out at the facility of the Horticulture and Pharmacognosy Field Station (Field Station for short), located at Downer's Grove, approximately 25 miles west of the University of Illinois campus in Chicago.

9.0 PERIOD AND PLAN OF RESEARCH

The proposed research will be carried out for a period of three years, beginning January, 1985.

During the first year of the project, emphasis will be given to herbarium and field works, and procurement of materials for phytochemical study. It is expected that by the end of the first year, the greater part of the inventory of the South American species of *Stevia* will have been completed. Chromosome counts, pollen examination with LM and SEM, flavonoid identification, sesquiterpene and diterpene glycoside screenings of a number of species will have been completed.

For the second year, study of herbarium material will continue, with special emphasis on the analysis of key characters and criteria for the subgeneric grouping of the South American species. During this period, work on flavonoid chemistry, diterpene glycosides and sesquiterpenes, pollen morphology and chromosome counts will reach a high level of intensity. By the end of the second year, it is expected that a complete inventory of all the South American species will have been completed and a preliminary scheme of classification and evolutionary trends will be identified. Field work will be carried out as scheduled.

During the third year, taxonomic study will continue, especially on nomenclature and in the construction of taxonomic keys and in species delimitation. Field work will be carried out as scheduled. It is expected that by the end of this year a complete list of accepted species of South American *Stevia* and keys of identification will have been completed, and data on chromosome number, pollen morphology, flavonoid, sesquiterpene, and diterpene glycoside chemistry will be available and ready to be assembled, in the reconstruction of the origin and evolution of *Stevia*.

10.0 DISSEMINATION OF RESEARCH RESULTS

The results of the proposed research will be made available to the scientific community through presentation in scientific meetings, publication in scientific journals, and seminar presentations in academic settings.

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BIOGRAPHICAL SKETCH (D. D. SOEJARTO)

Dr. D.D. Soejarto is an Associate Professor of Pharmacognosy in the PCRPS (Program for Collaborative Research in the Pharmaceutical Sciences), University of Illinois at Chicago, and a Research Associate of the Field Museum of Natural History (Botany), with a Ph.D. training in Systematic Botany (Harvard University, 1969).

He began his academic career as a Teaching Fellow in the Department of Biology, Harvard University, 1965-1967. Upon receiving his doctoral degree from Harvard, he went to Colombia, where he held an academic position in the Department of Biology, University of Antioquia (1969-1976). Since receiving his B.Sc. degree (1962, *cum laude*), Dr. Soejarto has been the recipient of several scholarships, i.e. Ford Foundation, Fernald Foundation, Ames Scholarship, and the Latin American Teaching Fellowship (Tufts University, 1969-1970). He has held the position Research Consultant of the Rockefeller Foundation (1974-1975) and the World Health Organization (WHO) (1978-1978), and member of the WHO Steering Committee (1976-1978) on the Indigenous Plants for Fertility Regulation Programme.

Dr. Soejarto has been involved in research and teaching in the plant sciences. At the University of Antioquia he has taught courses in general botany, morphology of vascular plants, plant microtechniques, taxonomy of vascular plants, and plant geography and evolution, and has taken part in a team teaching of courses in general ecology and economic botany. At his present appointment he is teaching a graduate level course in angiosperm taxonomy and phylogeny, and participating in a team teaching of pharmacognosy and chemotaxonomy. Although he has been actively involved in multidisciplinary research projects in the area of natural products and ethnobotany during the past eight years, he has continued with his taxonomic research, primarily on the genus Saurauia (Actinidiaceae).

Dr. Soejarto has an extensive botanical field work experience in the tropics, especially in tropical South America. He has participated in and carried out botanical collecting trips and expeditions in Ghana, Sri Lanka, Bangladesh, Thailand, Indonesia, Taiwan, Brazil, Paraguay, Bolivia, Peru, Ecuador, Colombia and Mexico, under the auspices of WHO and as part of his research involvement.

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BIOGRAPHICAL SKETCH (A.D. KINGHORN)

Dr. A.D. Kinghorn is an Associate Professor of Pharmacognosy at the Department of Medicinal Chemistry and Pharmacognosy, and an Associate Member of the PCRPS, University of Illinois at Chicago, with a Ph.D. training in Pharmacognosy (University of London, England, 1975).

Prior to studying for his Ph.D., Dr. Kinghorn was employed in industry, where he performed stability studies on formulated pharmaceuticals. He has taught in at the undergraduate level at the University of London and Illinois (natural product drug analysis), and graduate level courses in his present appointment on research techniques in pharmacognosy, phytochemistry, biosynthesis, chemotaxonomy, and the structure elucidation of natural products. Dr. Kinghorn was invited to organize both the 1977 and 1980 symposia of the Society for Economic Botany, and now serves as a Member of Council of this scientific group. He was an invited speaker at the International Legume Conference in London in July, 1978, and presented symposium lectures at the 43rd Federation Internationale Pharmaceutique meeting at Montreux, Switzerland, in September, 1983, and the second Australia-U.S. Conference on Poisonous Plants in Brisbane, Australia, in May, 1984. He has authored or co-authored over 65 research articles, has edited a book on poisonous plants, and has contributed a number of chapters to books on natural products.

Since receiving his Ph.D. in 1975, Dr. Kinghorn has conducted personal research and directed the research of post-doctoral associates, graduate students and research technologists. He has wide experience in the isolation, chemical analysis, identification, structure elucidation and bioassay of a broad range of natural products including sesquiterpenes, diterpenes, triterpenes, sterols, quassinoids, lignans, pyrones, flavonoids, coumarins, and various types of alkaloids and glycosides. His experience in extraction and separation techniques as applied to natural products includes the extensive use of gravity, low-pressure and high-pressure liquid chromatography (normal and reverse-phase), droplet counter-current chromatography, gas chromatography and thin-layer chromatography (analytical and preparative). He is experienced in the application of UV, IR, CD, proton NMR, carbon 13 NMR and mass spectrometry for the structure elucidation of isolates.

REPRESENTATIVE PUBLICATIONS (of more than 75)

In Press:

- Kinghorn, A.D., and D.D. Soejarto. 1984. "Current Status of Stevioside as a Sweetening Agent for Human Use." In: Progress in Medicinal and Economic Plant Research, Vol. 1, ed. H. Wagner, H. Hikino and N.R. Farnsworth, Academic, London.

Klocke, J.A., M. Arisawa, S.S. Handa, A.D. Kinghorn, G.A. Cordell and N.R. Farnsworth. 1984. Growth inhibitory, insecticidal and antifeedant effects of some antileukemic and cytotoxic quassinoids on two species of agricultural pests. Experientia.

Kinghorn, A.D., D.D. Soejarto, N.P.D. Nanayakkara, C.M. Compadre, H.C. Makapugay, J.M. Hovanec-Brown, P.J. Medon and S.K. Kamath. 1984. A phytochemical screening procedure for sweet ent-kaurene glycosides in the genus Stevia (Compositae). J. Nat. Prod.

Original Research Articles

1983 Soejarto, D.D., Compadre, P.J. Medon, S.K. Kamath and A.D. Kinghorn. 1983. Potential sweetening agents of plant origin. II. Field search for sweet-tasting Stevia species. Econ. Bot. 37:71-79.

Bhacca, N.S., M.F. Balandrin, A.D. Kinghorn, T.A. Frenkiel, R. Freeman and G.A. Morris. 1983. Carbon-13 and proton two-dimensional NMR study of the Ormosia alkaloids panamine, ormosanine, and ormosinine. J. Am. Chem. Soc. 105:2538-2544.

Lin, L.-J., G.T. Marshall and A.D. Kinghorn. 1983. The dermatitis-producing constituents of Euphorbia hermentiana Lem. Latex. J. Nat. Prod., 46:723-731.

Lin, L.-J. and A.D. Kinghorn. 1983. 8-Methoxyingol esters from the latex of Euphorbia hermentiana. Phytochemistry, 22:2795-2799.

Kinghorn, A.D. 1983. "Carcinogenic and Cocarcinogenic Toxins from Plants." In: Encyclopedic Handbook of Natural Toxins, vol. 1, Plant Toxins, eds. A.T. Tu and R.F. Keeler. New York, N.Y., Marcel Dekker Inc., 239-298.

Kinghorn, A.D. 1983. "New Plant-Derived Anticancer Drugs." In: Topics in Pharmaceutical Sciences 1983, eds. D.D. Breimer and P. Speiser, Elsevier Science Publishers, Amsterdam, Netherlands, 403-415.

1982 Kim, I.-C., M.F. Balandrin and A.D. Kinghorn. 1982. Reinvestigation of alkaloids of Lupinus sericeus Pursh. Identification of a new natural product, 10,17-dioxo-8-isosparteine. J. Agric. Food Chem. 30:796-798.

Balandrin, M.F. and A.D. Kinghorn. 1982. (-)-4 α -Hydroxysparteine, a new natural product from Acosmium panamense. Heterocycles 19:1931-1934.

Kinghorn, A.D., N.P.D. Nanayakkara, D.D. Soejarto, P.J. Medon and S.D. Kamath. 1982. Potential sweetening agents of plant origin. I. Purification of Stevia rebaudiana sweet constituents by droplet counter-current chromatography. J. Chromatogr. 237:478-483.

Soejarto, D.D., A.D. Kinghorn and N.R. Farnsworth. 1982. Potential sweetening agents of plant origin. III. Organoleptic evaluation of Stevia leaf herbarium samples for sweetness. J. Nat. Prod. 45:590-599.

1981 Balandrin, M.F. and A.D. Kinghorn. 1981. Characterization of sweetinine, a constituent of Sweetia elegans, as the Ormosia alkaloid, (+)-6-epipodopetaline. J. Nat. Prod. 44:619-622.

Hickey, T.A., S.M. Worobec, D.P. West and A.D. Kinghorn. 1981. Irritant contact dermatitis in humans from phorbol and related esters. Toxicol 19:841-850.

BIOGRAPHIC SKETCH (C.T. CHE)

Dr. Che is a Research Associate (phytochemistry) in the PCRPS (Program for Collaborative Research in the Pharmaceutical Sciences), University of Illinois at Chicago. He has more than eight years of experience in plant chemistry research, and is well-trained in the isolation, chemical analysis, identification, and structure elucidation of natural products. He is familiar with extraction and separation techniques such as gravity, low-pressure and high-pressure liquid chromatography, droplet counter-current chromatography, gas chromatography and thin-layer chromatography. He is also skilled in the use of UV, FT-IR, CD, proton NMR, carbon 13 NMR and mass spectrometry for the identification and structure elucidation of isolates.

Dr. Che has wide interests in natural products research. Prior to studying for his Ph.D., he worked on the chemical analysis of Chinese medicinal plants in Hong Kong, where he received a Master degree in Biochemistry. In 1982, he was granted his Ph.D. from the University of Illinois for work in the area of pharmacognosy. Shortly after obtaining his Ph.D., he joined the Ludwig Institute for Cancer Research (Toronto, Canada), as a post-doctoral fellow, to work on the chemical analysis of animal products and synthetic chemistry. In November, 1983, he rejoined the University of Illinois group to assume the position of Research Associate, and is presently involved in the isolation and identification of potential antitumor agents from plants.

PUBLICATIONS

In Preparation:

1. Cordell, G.A. et al. The X-ray crystallography of aristolindiquinone.
2. Che, C.T. et al. ¹H-NMR spectral analysis of some cucurbitacins.

In Press:

Che, C.T., M.S. Ahmed, S.S. Kang, D.P. Waller, A.S. Bingel, A. Martin, P. Rajamahendran, N. Bunyapraphatsara, D.C. Lankin, G.A. Cordell, D.D. Soejarto, R.O.B. Wijesekera & H.H.S. Fong. Studies on *Aristolochia*. III. Isolation and biological evaluation of constituents of *Aristolochia indica* L. roots for fertility-regulating activity. J. Nat. Prod.

Original Research Articles:

1983

Gupta, I., J. Baptista, W.R.Bruce, C.T.Che, R.Furrer, J.S.Gingerich, A.A.Grey, L.Marai, P.Yates, and J.J.Krepinsky. Structures of fecapentaenes, the mutagens of bacterial origin isolated from human feces. Biochemistry 22: 241-245.

Che, C.T., G.A.Cordell, H.H.S.Fong, and C.A.Evans. Aristolindiquinone, a new naphthoquinone from Aristolochia indica L. Tetrahedron Letters 24: 1333-1336.

1982

Bruce, W.R., J. Baptista, C.T.Che, R.Furrer, J.S.Gingerich, I.Gupta, and J.J.Krepinsky. General structure of fecapentaenes, the mutagenic substances in human feces. Naturwissenschaften 69: 557-558.

1980

Che, C.T., K.Koike, G.A.Cordell, H.H.S.Fong, and R.H.Dobberstein. Triterpenes of Pouteria torta. J. Nat. Prod. 43: 420-421.

1977

Kong, Y.C., C.T.Che, T.T.Yip, and H.M.Chang. Effects of Fructus Gardeniae extracts on hepatic functions. Comparative Medicine East and West 5: 241-255.

Che, C.T., T.T.Yip, K.H.Ng, and Y.C.Kong. Anti-hyperbilirubinemic effects of Fructus Gardeniae extracts. Planta Medica 32: 18-23.

1976

Kong, Y.C., S.Y.Hu, F.K.Lau, C.T.Che, H.W.Yeung, S.C.Cheong, and J.C.C.Hwang. Potential anti-fertility plants from Chinese Medicine. Amer. J. Chinese Med. 4: 105-128.

Kong, Y.C., F.K.Lau, and C.T.Che. A preliminary report on potential anti-fertility plants from Chinese medicine. J. of Chinese University of Hong Kong 3: 395-413.

APPENDIX III

SUMMARY
PROPOSAL BUDGET

First Year of Three Years

ORGANIZATION AND ADDRESS		FOR NSF USE ONLY	
PCRPS, UNIVERSITY OF ILLINOIS AT CHICAGO, 833 S. WOOD ST., CHICAGO, IL. 60612		PROPOSAL NO.	
PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR		DURATION (MONTHS)	
		PROPOSED	REVISED
A. SENIOR PERSONNEL (LIST BY NAME; SHOW NUMBERS OF PEOPLE IN BRACKETS; SALARY AMOUNTS MAY BE LISTED ON SEPARATE SCHEDULE) GPM 205.1b		NSF FUNDED MAN MONTHS CAL. ACAD. SUMR.	FUNDS REQUESTED BY PROPOSER
1. P.I./P.D. <u>Diaja Doel Soejarto, P.I. (30%)</u>		\$	—
2. CO P.I./P.D. <u>A. D. Kinghorn, Co-P.I. (5%)</u>		\$	—
3. CO P.I./P.D.		\$	
4. CO P.I./P.D.		\$	
5. CO P.I./P.D.		\$	
6. () SUBTOTALS A1 - A5		\$	
FACULTY AND OTHER SENIOR ASSOCIATES (ATTACH EXTRA SHEET IF NECESSARY)			
7. <u>Chun T. Che, Senior Inv. (100%)</u>		\$	25,000
8.		\$	
9.		\$	
10.		\$	
11.		\$	
12. () SUBTOTALS A7 - A11		\$	25,000
B. OTHER PERSONNEL (LIST NUMBERS IN BRACKETS)			
11141 1. () POSTDOCTORAL ASSOCIATES		\$	
11149 2. () OTHER PROFESSIONALS		\$	
11150 3. (2) GRADUATE STUDENTS (2 X 0.5 FTE)		\$	12,000
11152 4. () UNDERGRADUATE STUDENTS		\$	
11182 5. () SECRETARIAL - CLERICAL		\$	
11183 6. (1) TECHNICAL SHOP, OTHER (Part-time Gardener)		\$	2,856
TOTAL SALARIES AND WAGES (A+B)		\$	39,856
11200 C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS) @ 13.78%		\$	5,402
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A+B+C)		\$	45,348
D. EQUIPMENT (LIST ITEMS AND DOLLAR AMOUNTS FOR EACH ITEM)			
1 Phase-Contrast Microscope incl. a camera lucida attachment and camera adapter for microphotography \$4,000			
1 Rotavapor \$2,000			
23181 TOTAL EQUIPMENT		\$	6,000
E. MATERIALS AND SUPPLIES Reagents, solvents, glasswares, TLC plates, Chromat. papers, HPLC/GC columns plus accessories, micr. slides, photo supplies, plant collecting supplies		\$	5,000
32630 F. DOMESTIC TRAVEL Consultation visits to U.S. Herbaria and one scientific meeting per year		\$	800
42111 G. FOREIGN TRAVEL (LIST DESTINATION AND AMOUNT FOR EACH TRIP; GPM 731)			
Travels to carry out field work in Argentina, Paraguay, Bolivia, Peru (2-3 months)		\$	10,000
42112		\$	

SUMMARY PROPOSAL BUDGET

PAGE 2 OF 2 PAGES

BUDGET JUSTIFICATION

BUDGET A.

- A.1. No support is requested for the Principal Investigator (Dr. Soejarto), who will coordinate the overall operation of the proposed project, perform all aspects of the systematic work (incl. field work), and supervise graduate students (chromosome counts and pollen morphology).
- A.2. No support is requested for the Co-Principal Investigator (Dr. Kinghorn), who will coordinate the phytochemical aspects of the proposed project.
- A.7. A full support is requested for Dr. C. T. Che, who perform all aspects of the phytochemical investigation for the proposed project (flavonoid chemistry, diterpene glycoside screening, sesquiterpene survey) at 100% time. He will be in constant consultation with the Dr. A.D. Kinghorn.

BUDGET B.

- B.3. Support for two part-time graduate students (2 X 0.5-FTE) is requested. One student will be working on chromosome counts (incl. microphotography, camera lucida tracings), under the direction of Dr. Soejarto. The other student will be working on pollen morphology (incl. acetolysis, slide preparation, pollen measurements, microphotography), under the direction of Dr. Soejarto and Dr. Sylvia Feuer, a palynologist of the Field Museum of Natural History.
- B.6. Support is requested for a part-time gardener, who will be employed for cultivation experiments. His salary is based on a 21 hours of service per week (7 days) @ \$8/hr.

BUDGET D.

Fund is requested to purchase a phase-contrast microscope for cytological work. The Field Museum, where this work will be done, does not possess this type of microscope. A statement on the cost of this equipment from the Nikon dealer (Frank E. Fryer Co., Inc.) is enclosed (Appendix II). By prior agreement, this equipment will remain at the Field Museum at the conclusion of the proposed project.

Fund is also requested to purchase a Rotavapor for the exclusive use in phytochemical work of the proposed project. This equipment (Rotavapor R-110) is listed in the 1983 Fisher Scientific Cat. No. 09-548-151C.

BUDGET E.

Funds requested for this item include materials and supplies to perform work at the Field Museum (cytology, pollen morphology, loans of specimens) and at the University of Illinois (phytochemistry, SEM). One-fifth (\$1,000) of the budget requested corresponds to the Field Museum.

BUDGET F.

Domestic travels: Fund is requested to cover plane tickets and per diem for visits to several U.S. Herbaria and Libraries for consultation, such as Harvard University, the New York Botanical Garden, the U.S. National Herbarium.

Foreign travels: Fund is requested to cover field work expenses in South America and Mexico, which include plane tickets, vehicle rentals, vehicle insurance coverage, cost of gas to operate vehicles, mileage charges, cost of taxis, hotel accommodations, meals and lodgings (including expenses for a field assistant and/or collaborator), cost of collecting supplies and air shipment of plant materials (dried and liquid collections) from a foreign country to Chicago.

BUDGET N.

The indirect cost for the phase-contrast microscope and a portion (\$1,000) of the cost of the materials and supplies, for a total of \$5,000, is charged to the Field Museum of Natural History, which has an indirect cost rate of 80%. This indirect cost is included as a direct cost to the University of Illinois (Budget L).

allocated

APPENDIX III

SUMMARY
PROPOSAL BUDGET

Second Year of Three Years

ORGANIZATION AND ADDRESS		FOR NSF USE ONLY		
		PROPOSAL NO.		
PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR		DURATION (MONTHS)		
		PROPOSED	REVISED	
NSF USE	A. SENIOR PERSONNEL (LIST BY NAME; SHOW NUMBERS OF PEOPLE IN BRACKETS; SALARY AMOUNTS MAY BE LISTED ON SEPARATE SCHEDULE) GPM 209.10	NSF FUNDED MAN MONTHS CAL ACAD SUMR.	FUNDS REQUESTED BY PROPOSER	FUNDS GRANTED BY NSF (IF DIFFERENT)
	1. P.I./P.D. Djaja D. Soejarto, P.I. (30%)		\$ —	\$
	2. CO P.I./P.D. A.D. Kinghorn, Co-P.I. (5%)		\$ —	\$
	3. CO P.I./P.D.		\$	\$
	4. CO P.I./P.D.		\$	\$
	5. CO P.I./P.D.		\$	\$
11115	6. () SUBTOTALS A1 - A5		\$	\$
	FACULTY AND OTHER SENIOR ASSOCIATES (ATTACH EXTRA SHEET IF NECESSARY)			
	7. Chun T. Che, Senior Inv. (100%)		\$ 27,500	\$
	8.		\$	\$
	9.		\$	\$
	10.		\$	\$
	11.		\$	\$
11117	12. () SUBTOTALS A7 - A11		\$ 27,500	\$
	B. OTHER PERSONNEL (LIST NUMBERS IN BRACKETS)			
11141	1. () POSTDOCTORAL ASSOCIATES		\$	\$
11149	2. () OTHER PROFESSIONALS		\$	\$
11150	3. (2) GRADUATE STUDENTS (2 X 0.5 FTE)		\$ 13,200	\$
11152	4. () UNDERGRADUATE STUDENTS		\$	\$
11182	5. () SECRETARIAL - CLERICAL		\$	\$
11183	6. (1) TECHNICAL SHOP, OTHER (Part-time Gardener)		\$ 3,142	\$
	TOTAL SALARIES AND WAGES (A+B)		\$ 43,842	\$
11200	C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS) @ 13.78%		\$ 6,041	\$
	TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A+B+C)		\$ 49,883	\$
	D. EQUIPMENT (LIST ITEMS AND DOLLAR AMOUNTS FOR EACH ITEM)			
23181	TOTAL EQUIPMENT		\$	\$
	E. MATERIALS AND SUPPLIES Reagents, solvents, glasswares, TLC plates, Chromat. papers, HPLC/GC columns plus accessories, micr. slides, photo supplies, plant collecting supplies.		\$ 5,000	\$
32630			\$	\$
	F. DOMESTIC TRAVEL Consultation visits to US Herbaria and one scientific meeting per year		\$ 800	\$
42111			\$	\$
	G. FOREIGN TRAVEL (LIST DESTINATION AND AMOUNT FOR EACH TRIP; GPM 731) Travels to carry out field work in Brazil, Paraguay, Bolivia, Peru, Ecuador, Venezuela, Colombia (2-3 months)		\$ 10,000	\$
42112			\$	\$

SUMMARY PROPOSAL BUDGET

		PROPOSAL NO.
52500	H. PUBLICATION COSTS/PAGE CHARGES (incl. illustr +MS typing)	\$ 1,300
62315	I. COMPUTER (ADPE) SERVICES	\$
	J. CONSULTANT SERVICES (IDENTIFY CONSULTANTS BY NAME AND AMOUNT, GPM 518)	\$
	K. PARTICIPANT SUPPORT COSTS, IF ALLOWED BY PROGRAM GUIDE (ITEMIZE) GPM 518	\$
	1. STIPENDS	\$
	2. TRAVEL	\$
	3. SUBSISTENCE	\$
	4. OTHER - SPECIFY	\$
	5. TOTAL PARTICIPANT COSTS (K1 + K2 + K3 + K4)	\$
	L. ALL OTHER DIRECT COSTS (List items and dollar amounts. Details of subcontracts, including work statements and budget, should be explained in full in proposal.)	\$
	SEM filament time @ \$30/hr incl.	\$
	stubs, prep, gold coating, photo	\$ 2,000
	Field Museum indirect cost 80%x\$1000	\$ 800
65001	TOTAL OTHER DIRECT COSTS	\$ 2,800
	M. TOTAL DIRECT COSTS (A THROUGH L)	\$ 69,483
	N. INDIRECT COSTS (Specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs included in on/off campus bases in remarks.)	\$
74100	TOTAL INDIRECT COSTS	\$
	O. TOTAL DIRECT AND INDIRECT COSTS (M + N)	\$
74500	P. LESS RESIDUAL FUNDS (If for further support of current project; GPM 252 and 253)	\$
75000	Q. AMOUNT OF THIS REQUEST (O MINUS P)	\$

REMARKS

SALARY: salary increases are based on a projected 8% rate of increase over previous year.

FIELD MUSEUM indirect cost at the rate of 80% is charged to the portion of materials and supplies to be used in that institution, which is \$1,000 of the requested \$5,000.

NOTE: SIGNATURES REQUIRED ONLY FOR REVISED BUDGET (GPM 233). THIS IS REVISION NO.

SIGNATURE OF PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR	DATE OF SIGNATURE	TYPED OR PRINTED NAME AND TITLE
SIGNATURE OF AUTHORIZED ORGANIZATIONAL REPRESENTATIVE	DATE OF SIGNATURE	TYPED OR PRINTED NAME AND TITLE

FOR NSF USE ONLY

INDIRECT COST RATE VERIFICATION			PROGRAM OFFICER APPROVAL			
Date Checked	Date of Rate Sheet	Signature				
Grant Number	Agency	Institution	Organization	Fund Acct.	Program	Object
	Proposal Number	Dur.	Chg.	Award Date	Proposed Amount	Fund Dur.

APPENDIX III

SUMMARY
PROPOSAL BUDGET

Third Year of Three Years

ORGANIZATION AND ADDRESS		FOR NSF USE ONLY			
		PROPOSAL NO.		DURATION (MONTHS)	
PRINCIPAL INVESTIGATOR/PROJECT DIRECTOR		PROPOSED		REVISED	
		A. SENIOR PERSONNEL (LIST BY NAME; SHOW NUMBERS OF PEOPLE IN BRACKETS; SALARY AMOUNTS MAY BE LISTED ON SEPARATE SCHEDULE) GPM 205.1b		NSF FUNDED MAN MONTHS CAL. ACQA. SUMR.	
1. P.I./P.D. Djaja Doel Soejarto, P.I. (30%)				\$	\$
2. CO P.I./P.D. A.D. Kinghorn, Co-P.I. (5%)				\$	\$
3. CO P.I./P.D.				\$	\$
4. CO P.I./P.D.				\$	\$
5. CO P.I./P.D.				\$	\$
6. () SUBTOTALS A1 - A5				\$	\$
FACULTY AND OTHER SENIOR ASSOCIATES (ATTACH EXTRA SHEET IF NECESSARY)					
7. Chun T. Che, Senior Inv. (100%)				\$ 30,350	\$
8.				\$	\$
9.				\$	\$
10.				\$	\$
11.				\$	\$
12. () SUBTOTALS A7 - A11				\$ 30,250	\$
B. OTHER PERSONNEL (LIST NUMBERS IN BRACKETS)					
11141 1. () POSTDOCTORAL ASSOCIATES				\$	\$
11149 2. () OTHER PROFESSIONALS				\$	\$
11150 3. (2) GRADUATE STUDENTS (2 X 0.5 FTE)				\$ 14,520	\$
11152 4. () UNDERGRADUATE STUDENTS				\$	\$
11182 5. () SECRETARIAL - CLERICAL				\$	\$
11183 6. (1) TECHNICAL SHOP OTHER (Part-time Gardener)				\$ 3,456	\$
TOTAL SALARIES AND WAGES (A+B)				\$ 48,226	\$
11200 C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS) @ 13.78%				\$ 6,646	\$
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A+B+C)				\$ 54,872	\$
D. EQUIPMENT (LIST ITEMS AND DOLLAR AMOUNTS FOR EACH ITEM)					
23181 TOTAL EQUIPMENT				\$	\$
E. MATERIALS AND SUPPLIES Reagents, solvents, glasswares, TLC plates, Chromat. papers, HPLC/GC columns, plus accessories, micr. slides, photo supplies, plant collecting supplies.				\$ 5,000	\$
32630 F. DOMESTIC TRAVEL Consultation visits to US Herbaria and one scientific meeting per year				\$ 800	\$
42111 G. FOREIGN TRAVEL (LIST DESTINATION AND AMOUNT FOR EACH TRIP; GPM 731) Travels to carry out field work in Brazil, Bolivia, Mexico (approximately 2 months)				\$	\$
42112				\$ 6,000	\$

Third Year of Three Years

PAGE 2 OF 2 PAGES

APPENDIX IV

Current and Pending Support

The following information should be provided for each investigator and other senior personnel (see p. 7). Failure to provide this information may delay consideration of the proposal.

	A	B	C	D	E	F
	Supporting Agency	Project Title	Award Amount (or Annual Rate)	Period Covered By Award	Man-Months Or % of Effort Committed To The Project	Location Where Research Is/Will Be Performed
ACAD. SUMM.						
I. (Name of Principal Investigator)	Djaja Doel Soejarto					
A. Current Support						
List—If none, Report none	1,2,3	1,2,3	1,2,3	1,2,3	1,2,3--	1,2,3
B. Proposals Pending						
1. List this proposal	4	4	4	4	4	4
2. Other pending proposals, including renewal applications	5	5	5	5	5	5
3. Proposals planned to be submitted in near future	--	--	--	--	--	--
II. (Name of co-principal investigator and/or faculty associate)	A. Douglas Kinghorn					
A. Current Supports	6,7	6,7	6,7	6,7	6,7	6,7
B. Proposals Pending	8,9	8,9	8,9	8,9	8,9	8,9
III. Transfer of Support						
If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.	--	--	--	--	--	--
IV. (Other agencies to which this proposal has been/will be submitted)	--					

Please see p. 40 for the meaning of the codes 1 - 9.

CURRENT AND PENDING SUPPORTS

Explanation for Codes

Code Source of Funding

D.D. Soejarto

- 1 World Health Organization. Funded.
Project Title: Multicentre Collaborative Approach for the Isolation of Fertility Regulating Agents from Plants.
Award Amount: \$160,000.
Period Covered: 1 Jan. - 31 Dec., 1984. (Renewable).
Percent Effort: 10%.
Role: Co-P.I. (P.I.: H.H.S. Fong.)
Location: PCRPS.
- 2 National Science Foundation. Funded.
Project Title: NAPRALERT, A Specialized Data Base on Natural Products.
Award Amount: \$275,000.
Period Covered: 1 Jan., 1984 - 31 Dec., 1986.
Percent Effort: 15%.
Role: Co-P.I. (P.I.: N.R. Farnsworth.)
Location: PCRPS.
- 3 National Cancer Institute. Funded.
Project Title: Literature Surveillance of Natural Products.
Award Amount: \$355,555.
Period Covered: 1 Jan., 1983 - 31 Dec., 1986.
Percent Effort: 15%.
Role: Co-P.I. (P.I.: W.D. Loub.)
Location: PCRPS.
- 4 National Science Foundation. Pending.
Project Title: Studies of South American Stevia (Compositae - Eupatorieae).
Amount Requested: \$207,403.
Period Covered: 1 Jan., 1985 - 31 Dec., 1987.
Percent Effort: 30%.
Role: Principal Investigator.
Location: PCRPS and Field Museum of Natural History.
- 5 National Institute of Dental Research. Pending.
Project Title: Potential Non-Cariogenic Sweetening Agents from Plants.
Amount Requested: \$248,490.
Period Covered: 1 Spet., 1984 - 31 Aug., 1987.
Percent Effort: 10%.
Role: Co-P.I. (P.I.: A.D. Kinghorn.)
Location: PCRPS.

A.D. Kinghorn

- 6 National Cancer Institute. Funded.
Project Title: Plant Anticancer Agents: Isolation and Evaluation.
Award Amount: \$316,577.
Period Covered: 1 July, 1983 - 30 June, 1988.
Percent Effort: 15%.
Role: Co-P.I. (P.I.: N.R. Farnsworth.)
Location: PCRPS.
- 7 NIOSH. Funded.
Project Title: Measurement of Hypersensitivity Responses to Poison Ivy.
Award Amount: \$14,186.
Period Covered: 1 July, 1983 - 30 June, 1984.
(Non-renewable.)
Percent Effort: 10%.
Role: Co-P.I. (P.I.: S.M. Worobec.)
Location: PCRPS.
- 8 National Science Foundation. Pending.
Project Title: Studies of South American Stevia (Compositae - Eupatorieae).
Amount Requested: \$207,403.
Period Covered: 1 Jan., 1985 - 31 Dec., 1987.
Percent Effort: 5%.
Role: Co-P.I. (P.I.: D. D. Soejarto.)
Location: PCRPS.
- 9 National Institute of Dental Research. Pending.
Project Title: Potential Non-Cariogenic Sweetening Agents from Plants.
Amount Requested: \$248,490.
Period Covered: 1 Sept., 1984 - 31 Aug., 1987.
Percent Effort: 20%.
Role: P.I.
Location: PCRPS.

APPENDIX I

LIST OF SPECIES OF SOUTH AMERICAN STEVIA AT F¹

Species	Origin of Specimen(s)
<i>S. achalensis</i> Hieron.	Argentina
<i>S. alpina</i> Griseb.	Argentina
<i>S. alternifolia</i> Hieron.	Brazil
<i>S. amambayensis</i> B.L. Robins.	Paraguay
<i>S. ammotropha</i> B.L. Robins.	Paraguay
<i>S. amplexicaulis</i> Hassler	Paraguay
<i>S. andina</i> B.L. Robins.	Peru
<i>S. anisostemma</i> Turcz.	Ecuador
<i>S. apensis</i> B.L. Robins.	Paraguay
<i>S. aristata</i> D. Don	Argentina, Paraguay
<i>S. bangii</i> Rusby	Bolivia
<i>S. benderi</i> Perk.	Bolivia
<i>S. benthamiana</i> Hieron.	Bolivia
<i>S. bermejensis</i> Hieron.	Bolivia
<i>S. bertholdii</i> B.L. Robins.	Ecuador
<i>S. boliviensis</i> Sch.-Bip.	Bolivia
<i>S. breviaristata</i> Hook. & Arn.	Argentina
<i>S. brevipapposa</i> Hieron.	Bolivia
<i>S. burkartii</i> B.L. Robins.	Argentina
<i>S. calabambensis</i> Hieron.	Peru
<i>S. caldillerensis</i> Hieron.	Bolivia
<i>S. calycina</i> DC.	Brazil
<i>S. camachensis</i> Hieron.	Bolivia
<i>S. camporum</i> Baker	Brazil
<i>S. cardiatica</i> Perk.	Bolivia
<i>S. cathartica</i> Poepp.	Ecuador, Peru
<i>S. chamaedrys</i> Griseb.	Argentina
<i>S. cinerascens</i> Sch.-Bip.	Brazil
<i>S. clausenii</i> Sch.-Bip.	Brazil
<i>S. clivicola</i> B.L. Robins.	Bolivia
<i>S. cochabambensis</i> Hieron.	Bolivia
<i>S. collina</i> Gardn.	Brazil
<i>S. compacta</i> Benth.	Bolivia, Colombia
<i>S. commixta</i> B.L. Robins.	Brazil
<i>S. crenata</i> Benth.	Ecuador
<i>S. crenulata</i> Baker	Brazil
<i>S. cuneata</i> Hassler	Paraguay
<i>S. cuzcoensis</i> Hieron.	Peru
<i>S. decussata</i> Baker	Brazil
<i>S. dianthoidea</i> Hieron.	Ecuador
<i>S. dissecta</i> B.L. Robins.	? Peru

¹All the species listed here are represented by herbarium specimens and photographs at the John G. Searle Herbarium, Field Museum of Natural History, Chicago. The Latin binomials appearing in this list are those as annotated on the mounted specimens and/or photographs.

Species	Origin of Specimen(s)
<i>S. diversipapposa</i> Hieron.	Argentina
<i>S. dubia</i> B.L. Robins.	Brazil
<i>S. effusa</i> Hieron.	Argentina
<i>S. elatior</i> HBK.	Colombia, Ecuador, Venezuela
<i>S. elongata</i> HBK.	Colombia, Venezuela
<i>S. entrerriensis</i> Hieron.	Argentina, Paraguay, Uruguay
<i>S. estrellensis</i> Hassler	Paraguay
<i>S. fastigiata</i> HBK.	? Colombia
<i>S. fiebrigii</i> Hieron.	Bolivia
<i>S. filipes</i> Rusby	Bolivia
<i>S. galeopsidifolia</i> Hieron.	Peru
<i>S. gilliesii</i> Hook. & Arn.	Argentina, Brazil
<i>S. glanduloso-pubescens</i> Hieron.	Bolivia
<i>S. glomerata</i> Hieron.	Bolivia
<i>S. grisebachiana</i> Hieron.	Argentina
<i>S. guaranitica</i> Chodat	Paraguay
<i>S. hassleriana</i> Chodat	Paraguay
<i>S. heptachaeta</i> DC.	Brazil
<i>S. herrerae</i> B.L. Robins.	Peru
<i>S. hirtiflora</i> Sch.-Bip.	Venezuela
<i>S. hypericifolia</i> Hieron.	Brazil
<i>S. hyptifolia</i> Gardn.	Brazil
<i>S. hyssopifolia</i> Philippi	Chile
<i>S. involucrata</i> Sch.-Bip.	Brazil
<i>S. kuntzei</i> Hieron.	Bolivia
<i>S. lehmannii</i> Hieron.	Colombia
<i>S. leptophylla</i> Sc.-Bip.	Paraguay
<i>S. leucosticta</i> B.L. Robins.	Peru
<i>S. lilloi</i> B.L. Robins.	Argentina
<i>S. linariaefolia</i> DC.	Brazil
<i>S. lorentzii</i> Griseb.	Argentina
<i>S. lucida</i> Lag.	Colombia, Venezuela
<i>S. lundiana</i> DC.	Brazil
<i>S. macbridei</i> B.L. Robins.	Peru
<i>S. mandonii</i> Sch.-Bip.	Bolivia, Peru
<i>S. megapotamica</i> DC.	Brazil
<i>S. melissaeifolia</i> Sch.-Bip.	Peru
<i>S. menthaefolia</i> Sch.-Bip.	Brazil, Chile
<i>S. mercedensis</i> Hieron.	Argentina
<i>S. microcephala</i> DC.	Peru
<i>S. microphylla</i> HBK.	? Colombia
<i>S. minor</i> Griseb.	Argentina
<i>S. multiaristata</i> Spreng.	Argentina, Brazil, Uruguay
<i>S. myriadenia</i> Sc.-Bip.	Brazil
<i>S. nepetaefolia</i> Willd.	Colombia
<i>S. nevadensis</i> Hieron.	Argentina
<i>S. obovata</i> Rusby	Bolivia
<i>S. neglecta</i> Rusby	Bolivia
<i>S. oligocephala</i> DC.	Brazil

Species	Origin of Specimen(s)
<i>S. ophryophylla</i> B.L. Robins.	Brazil
<i>S. organensis</i> Gardn.	Brazil
<i>S. ovata</i> Willd.	Brazil
<i>S. oxylaena</i> DC.	Brazil, Uruguay
<i>S. pabloensis</i> Hieron.	Peru
<i>S. pallida</i> (Sch.-Bip.) Hieron.	Nenezela
<i>S. pauciradiata</i> Baker	Brazil
<i>S. parviflora</i> Hassler	Paraguay
<i>S. pennellii</i> B.L. Robins	Peru
<i>S. petiolata</i> (Cass.) Sch.-Bip.	Peru
<i>S. philippiana</i> Hieron.	Chile
<i>S. pohliana</i> Baker	Brazil
<i>S. potrerensis</i> Hieron.	Argentina
<i>S. procumbens</i> Hieron.	Argentina
<i>S. puberula</i> Hook.	Peru
<i>S. pubigera</i> Hieron.	Argentina
<i>S. purdiei</i> B.L. Robins.	Colombia
<i>S. purpurea</i> HBK.	Ecuador
<i>S. quitensis</i> HBK.	Ecuador
<i>S. rebaudiana</i> Bertoni	Paraguay
<i>S. regnellii</i> Sch.-Bip.	Brazil
<i>S. rhombifolia</i> HBK.	Col., Ec., Peru, Ven.
<i>S. riedelii</i> Sch.-Bip.	Brazil
<i>S. rojasii</i> Hassler	Paraguay
<i>S. saltenis</i> Hieron.	Argentina
<i>S. sanguinea</i> Hieron.	Argentina
<i>S. santacruzensis</i> Hieron.	Bolivia
<i>S. satureioides</i> Sch.-Bip.	Argentina
<i>S. satureiaefolia</i> Sch.-Bip.	Argentina, Brazil, Uruguay
<i>S. schickendantzii</i> Hieron.	Argentina
<i>S. schreiteri</i> B.L. Robins.	Argentina
<i>S. schultzii</i> Hieron.	Bolivia
<i>S. selloi</i> Spreng.	Brazil, Paraguay
<i>S. serrata</i> Cav.	Colombia, Venezuela
<i>S. soratensis</i> Hieron.	Bolivia, Peru
<i>S. stubelii</i> Hieron.	Bolivia
<i>S. tapacariensis</i> Hieron.	Bolivia
<i>S. tarijensis</i> Hieron.	Bolivia
<i>S. tenuifolia</i> Philippi	Chile
<i>S. triaristata</i> Hieron.	Bolivia
<i>S. tunguraguensis</i> Hieron.	Ecuador
<i>S. urticaefolia</i> Billb.	Brazil, Bolivia
<i>S. vaga</i> Griseb.	Argentina
<i>S. veronicae</i> DC.	Brazil, Paragua
<i>S. wagneri</i> Hieron.	Colombia, Venezuela
<i>S. weberbaueri</i> B.L. Robins.	Peru
<i>S. yaconensis</i> Hieron.	Argentina, Bolivia

APPENDIX II

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THE
UNIVERSITY
OF
ILLINOIS
AT
CHICAGO

Department of Biological Sciences
College of Liberal Arts and Sciences
Box 4348, Chicago, Illinois 60680
(312) 996-2211

March 30, 1984

Dr. Doel Soejarto
College of Pharmacy
833 S. Wood St.
University of Illinois at Chicago
Chicago, IL 60612

Dear Dr. Soejarto:

In response to your letter of March 26, 1984, the current cost for SEM work for departments at the University of Illinois at Chicago is \$30.00 per filament hour. This price also includes limited specimen preparation (freeze-drying or critical point drying), mounting of specimens on stubs, gold coating, and a maximum of 10 P/N micrographs per hour.

The SEM work would be carried out on a high resolution ISI-DS-130 scanning electron microscope.

Your truly,

Ron Wibel
SEM Technologist

RW:gt

**FRANK E. FRYER COMPANY, INC.**

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March 27, 1984

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COLL.

Robert Conlin

Delivered

to Djaja SoejartoAssoc. Professor of PharmacognosyUniversity of Illinois of ChicagoCollege of Pharmacy833 S. WoodChicago, IL 60612 312-996-8889

We are pleased to submit the following quotation.

QUANTITY	DESCRIPTION	PRICE	AMOUNT
	NIKON LABOPHOT MICROSCOPE		
1	#F79001 Nikon Labophot U.L. Listed stand with reverse position nosepiece, in-base high-intensity 6V/20W tungsten halogen Koehler illumination system, in-base transformer with continuously variable solid-state integrated circuit intensity control, grounded 3-wire power cord. Low-position coaxial coarse and fine focus assembly on roller-bearing slide-ways. Fine adjustment is graduated in 2 micrometer units. Coarse and fine adjustment have 30mm travel. Centerable substage condenser carrier on rack-and-pinion slideway dovetail slider for rapid interchange of condensers, 5-place turret nosepiece with ball-bearing motion. Internal clickstop with No. 79099 6V/20W bulb.		\$ 554.00
1	#F79005 "F" Trinocular body with high-transmission multilayer coatings; rotatable 360°, constant tube length Sidentopf binocular inclined 30° from horizontal with graduated interpupillary adjustment from 51mm to 75mm; vertical monocular phototube; swing-out prism for 100% visual or 100% photo. Mag. factor 1x.		428.00
1	#F79010 Rectangular mechanical stage, low position, short stage finger, right hand, coaxial controls, with 180mm x 140mm surface, 76mm x 50mm travel; removable slide holder.		185.00

QUOTE VALID FOR _____ DAYS.

BY

Robert Conlin



FRANK E. FRYER COMPANY, INC.

36 E. MAIN STREET CARPENTERSVILLE, ILLINOIS 60110 312/426-6731

TO <u>Djaja Soejarto</u>		DATE <u>March 27, 1984</u>	# <u>013680</u>	Please indicate this number when ordering
Assoc. Professor of Pharmacognosy		YOUR INQUIRY DATED <u>Demo/Andre</u>	TERMS <u>net 30</u>	
University of Illinois at Chicago		PROPOSED SHIPPING DATE	TO BE SHIPPED VIA	
College of Pharmacy		<u>Stock</u>	<u>best way</u>	
<u>833 S. Wood</u>		SALESMAN	F.O.B.	PPD. COLL.
<u>Chicago, IL 60612</u>		<u>Robert Conlin</u>	<u>Delivered</u>	

We are pleased to submit the following quotation.

QUANTITY	DESCRIPTION	PRICE	AMOUNT
2	#F79040 CFDW 10x widefield eyepieces	81.00ea	\$ 162.00
1	#F79111 Abbe phase turret condenser, n.a. 1.25, with centering screws and phase annuli for 10x, 20x, 40x and 100x; dry darkfield annulus for 10x, 20x, and 40x; brgithfield position with aperture diaphragm; centering telescope, interference filter 546mm (green) and heat absorbing filter/		355.00
1	#F78805 CF DL 10x Phase contrast, plan achromat		359.00
1	#F78806 CF DL 20x Phase contrast plan achromat		387.00
1	#F78813 DM 40x CF Phase contrast plan achromat		221.00
1	#F78814 DM 100x CF Phase contrast plan achromat		360.00
1	#F79420 Simple photo adapter		54.00
1	#F79410 PL 2.5x projection lens		113.00
1	#F2360 F to C Adapter		87.50
TOTAL			\$3,265.50

QUOTE VALID FOR _____ DAYS.

BY

Robert Conlin

APPENDIX III

FACILITIES

UNIVERSITY OF ILLINOIS AT CHICAGO: PCRPS

1. Space

PCRPS is located on the third and fourth floor of the College of Pharmacy building, University of Illinois at Chicago. Floor plans are illustrated in Figs. 4 and 5. For the conduct of the proposed research, the following space and facilities will be available for use:

Room 304 J : This is the office of the Principal Investigator, covering an area of approximately 200 sq. ft.

Room 310 : This room is the PCRPS's secretarial office, covering an area of 400 sq. ft.

Room 348 : This is a general phytochemistry laboratory with walk-in and standard hoods, covering a total area of 930 sq. ft. and fitted with three laboratory benches. Dr. C. T. Che has been assigned to one of the benches.

Room 350 : This is the chromatography laboratory, covering an area of 480 sq. ft. Most major chromatographic equipments are located here (See Table 1).

Room 452 : This is the office-laboratory of the Co-Principal Investigator, covering an area of 250 sq. ft.

2. Equipments

Equipments that will be available for the conduct of the proposed project are listed in Tables 1-2.

3. Library of the Health Sciences

The Campus library, Library of the Health Sciences, is located immediately adjacent to the PCRPS laboratories. The facility is staffed by 30 professional personnel, and receives over 3,400 periodicals. In addition to more than 500,000 bound books, periodicals and multimedia items, the library provides access to about 100 on-line data files.

FIELD MUSEUM OF NATURAL HISTORY (BOTANY DEPARTMENT)

The Botany Department is located on the third floor of the Field Museum of Natural History. The Herbarium of the Botany Department (the John G. Searle Herbarium) has a holding of 2 million mounted specimens, whose strength is the flora of tropical America. The Botany Department library has a holding

of more than 40,000 botanical titles. This library is supported by the main library, which houses all other natural history titles, reference works, and rare books.

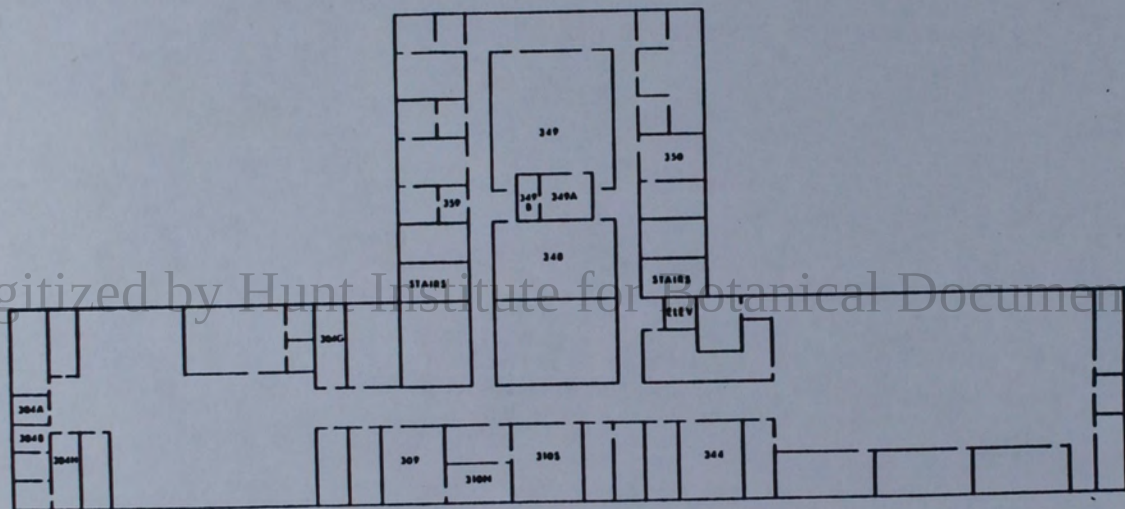
The Principal Investigator has been assigned an office-laboratory, covering an area of 260 sq. ft., equipped with herbarium cases, work benches, cabinets, and a herbarium dissecting microscope.

The Botany Department is also equipped with a laboratory for plant morphology and palynology, covering an area of 400 sq. ft. This laboratory is fitted with working benches, drawer cabinets, and a laminar-flow hood. The following equipments housed in this laboratory will be available for use for the proposed project: Paraffin oven, centrifuges, dissecting and compound microscopes (palynology), microscope-slide dryer, and a polaroid camera set-up.

HORTICULTURE AND PHARMACOGNOSY FIELD STATION, UNIVERSITY OF ILLINOIS, DOWNER'S GROVE, ILLINOIS.

The Field Station comprises a 50-acre site, of which 10 acres are owned by the University, the other 40 being leased. On the 10-acre piece of land are located a medicinal plant garden and two large greenhouses connected by a buffer area for office, workshop, a garage and a storage area. Other buildings on this land are a shed to store bulk plant samples, an extraction cabin, and a quarter for animal breeding. The remaining 40-acre land consists of open meadows and wooded lands, interrupted by two ponds and a horticulture display area, and is used for the cultivation of large quantities of research material. Both the greenhouses and the outdoor cultivation areas will be available for use for the proposed project. Fig. 6 presents the physical plan of the Field Station.

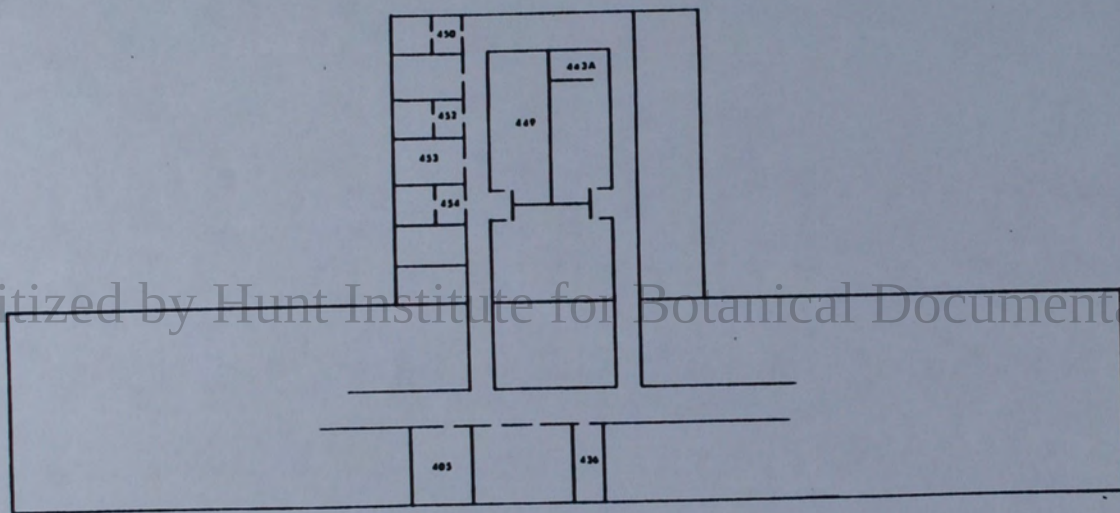
The Field Station is staffed by a foreman, an electrical engineer, and a temporary assistant. An additional part-time assistant will be hired for the proposed project.



THIRD FLOOR PLAN

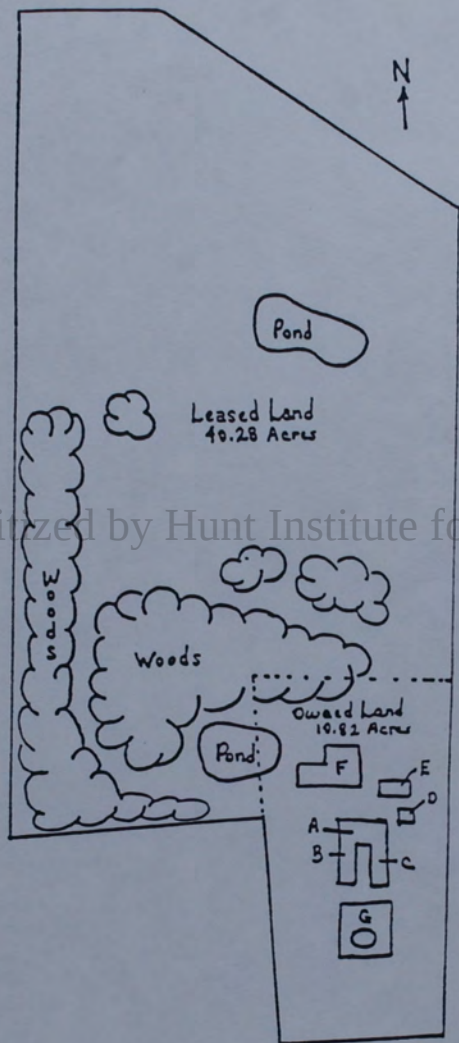
Figure 4.

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FOURTH FLOOR PLAN

Figure 5.



<u>Loc.</u>	<u>Facility</u>
A	Head House (with grinding Room)
B	Greenhouse
C	Greenhouse
D	Extraction Bldg.
E	Storage Garage
F	Rabbit house
G	Botanical Garden

Figure 6. Physical Plan of the Horticulture and Field Station of the University of Illinois at Downer's Grove.

Table 1. Analytical and Spectroscopic Equipment That Will Be Available For the Conduct of The Proposed Research

Quantity	Item	Location in the College Pharm Bldg
1	Gas Chromatography, Perkin-Elmer Model 3920	Room 350
1	High Pressure Liquid Chromatographic System, Waters Associates, Model 6000A pump and Model 440 UV Detector	Room 350
1	High Pressure Liquid Chromatographic System, Beckman, Model 421 System Controller and Model 100A and 110A pumps, Perkin-Elmer, Model LC-85 Variable Wavelength UV detector Beckman, Model C-RIA printer plotter integrator	Room 350
1	High Pressure Liquid Chromatographic Columns, various packings and particle size	Room 350
1	Melting Point Apparatus, Thomas-Hoover	Room 350
1	Bentam Ware Kit #4, Kontes, plus extra micro accessories	Room 348
3	Balances, top-loading, automatic, Mettler P1200	Room 348
3	Balances, analytical, automatic Mettler H20T, Mettler Gram-matic, Ainsworth SC	Room 348
1	Pump for low pressure column chromatography, Milton Roy	Room 348
1	Low Pressure Liquid Chromatography Column Kit, Ace Glass	Room 350
1	Infrared Spectrophotometer, Nicolet, Model MX-1 Fourier Transform	Room 554
1	Infrared Spectrophotometer, Beckman, Model IR-18A	Room 350

- | | | |
|---|--|--|
| 1 | Ultraviolet Spectrophotometer,
Beckman, Model DB-G | Room 350 |
| 1 | NMR Spectrophotometer, Varian,
Model T-60A (60 MHz) with a
Nicolet, Model TT-7 Fourier
Transform attachment, Probes
for both ¹ H and ¹³ C NMR are
available | Room 554 |
| 1 | Optical Rotary Dispersion/Circular
Dichroism Instrumnt, JASCO, Model
J-40 | Room 554 |
| 1 | Mass Spectrometer, Varian MAT,
Model 112S (double focussing
high resolution) with SS-166 Data
System and Varian, Model 1400,
Gas Chromatograph | Room E-20 of the
Research Resources
Center (RRC) |
| 1 | NMR Spectrometer, Nicolet, Model
NC360NB (360 MHz), FT, ¹ H, ¹³ C/
² H capability | Room E-9-11 of RRC |
| 1 | Mass Spectrometer, Finnegan MAT
Model 4500, with GC attachment
and FAB module | Clin Pharmacokin.
Lab. |

Table 2. Phytochemical Isolation Equipment

Quantity	Item	Location in the College Pharm Bldg
30	Chromatography Columns, assorted sizes up to 11 cm in diameter	Rooms 304H, 348
30	Separatory funnels, assorted (1 - 6 liters) size	Rooms 348, 349
3	Flash Evaporators, Buchi, Model R continuous	Room 348
1	Preparative High Pressure Liquid Chromatograph, Jobin Yvon, Chromatospac 10/100 with Gow Mac 80/800 Preparative LC/UV detector and recorder	Room 350
1	Droplet Counter Current Chromatograph, Tokyo Rikakikai, Model DCC-A	Room 350
1	Automatic fraction collector, Buchler, Fractomette Alpha 200	Room 350
1	Paper Chromatography Chamber, large size	Room 350
3	UV-Visible light viewing boxes Chromato-vue	Room 348
5	TLC tanks	Room 348

A PHYTOCHEMICAL SCREENING PROCEDURE FOR SWEET *ENT*-KAURENE
GLYCOSIDES IN THE GENUS *STEVIA* (COMPOSITAE)^{1,2}

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A.D. Kinghorn, D.D. Soejarto³, N.P.D. Nanayakkara, C.M. Compadre,
H.C. Makapugay, J.M. Hovanec-Brown, P.J. Medon⁴ and S.K. Kamath⁵

Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy
Health Sciences Center, University of Illinois at Chicago, Chicago,
Illinois 60612.

In Press: J. Nat. Prod. (1984).

Abstract. - Altogether 110 species of the genus *Stevia*, comprising both herbarium and fresh leaf samples, were screened for the presence of sweet *ent*-kaurene glycosides, using a combination of thin-layer and high-pressure liquid chromatography, followed by gas chromatography/mass spectrometry. Stevioside and rebaudiosides A and C were detected in a *S. rebaudiana* herbarium specimen collected in Paraguay in 1919, and stevioside was observed as a constituent of a *S. phlebophylla* herbarium specimen collected in Mexico in 1889. Steviol glycosides were not detected in any of the other 108 *Stevia* species studied. The phytochemical results obtained in this study are correlated with those of preliminary organoleptic tests on the sweetness of these *Stevia* samples, and the chemotaxonomic implications of the present findings are discussed.

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In preliminary studies designed to investigate the distribution of sweet *ent*-kaurene glycosides in the genus *Stevia*, we have studied organoleptically 184 *Stevia* leaf samples taken from an herbarium (1), as well as the leaves of 31 species of this genus collected in the field (2). Although a number of stored and fresh *Stevia* leaves exhibited taste sensations that were perceived as being slightly sweet, none approached the intense sweetness sensations produced by leaves of *Stevia rebaudiana* (Bertoni) Bertoni, as demonstrated by both a specimen preserved for over 60 years, as well as a live specimen collected in its native habitat in northeastern Paraguay (1,2).

Despite the current commercial use of *S. rebaudiana* products in Japan and Brazil for sweetening purposes (1), and the fact that many *Stevia* species are used medicinally (3), only about 20 members of this large genus have been studied phytochemically to date. Thus far, none of the eight sweet *ent*-kaurene glycoside constituents of *S. rebaudiana*, namely, dulcoside A, rebaudiosides A-E, steviolbioside and stevioside, have been reported as constituents of any other *Stevia* species. However, a group of non-sweet *ent*-kaurene glycosides, paniculosides I-V, have been reported to occur in *S. paniculata* Lag. (4) and *S. ovata* Lag. (5) leaves. Other phytochemical papers have reported in *Stevia* species bisabolane (6-9), germacrane (6-8,10-12), guaiane (8,10,13,14), humulane (7,10), α -longipinane (6,7,10,15) and pseudoguaiane (16) sesquiterpene derivatives, clerodane (8,17), kaurene (8,10,18) and labdane (6,8,18-20) diterpene derivatives, as well as sterols (10,18,20,21), triterpenes (10,20), flavonoids (6,19,21,22), chromenes (6,18,23), euparine derivatives (10), and miscellaneous volatile constituents (7,8,21,24,25). Phytochemical screening studies to determine the infrageneric distribution of either *ent*-kaurene glycosides or any of the other known *Stevia* constituents are so far lacking, thus the chemotaxonomic significance of all of the secondary metabolites so far isolated from plants in this genus is presently unknown.

In the present paper we describe a phytochemical screening method to detect sweet steviol (13-hydroxy-*ent*-kaurenoic acid) glycoside constituents in 184 *Stevia* herbarium samples (110 species, 121 taxa), and seven of these species that were collected in the field in Peru and Mexico. Such a study was initiated in an attempt to locate new sources of sweet *ent*-kaurene glycosides within the genus *Stevia*.

RESULTS AND DISCUSSION

The sweet compound stevioside was detected by analytical tlc and hplc in leaf herbarium samples of *S. rebaudiana* (Bertoni) Bertoni, collected in Paraguay in 1919 (Gosling s.n.) and *S. phlebophylla* A. Gray, collected in Mexico in 1889 (Pringle 2291). The additional sweet compounds, rebaudiosides A and C, were detected in the *S. rebaudiana* sample. These observations confirm our previous assertion of the sweet nature of these same samples after organoleptic evaluation (1). None of the above-mentioned compounds, nor any of the other known sweet *ent*-kaurene constituents of *S. rebaudiana* were identified in leaf samples of the remaining 108 *Stevia* species investigated.

Before commenting on the implications of these results, it is pertinent to briefly discuss the methodology described here for the purpose of phytochemical screening of *Stevia* species for the presence of sweet *ent*-kaurene glycosides. The positive glycoside identifications proposed for the *S. rebaudiana* and *S. phlebophylla* samples were based on a combination of chromatographic data, owing to the small amounts of plant material available and the consequent impracticability of isolating these sweet principles. However, the specificity of such compound identification was increased by the unequivocal generation of isosteviol methyl ester from both plants by acid hydrolysis and methylation, as demonstrated by gc/ms. Isosteviol, a known product of stevioside on acid hydrolysis (26,27), was also shown here to be produced after reflux of rebaudiosides A-E, dulcoside A and steviolbioside, a treatment undergone by all of the *Stevia* samples examined

in this study. The failure to detect isosteviol on acid hydrolysis of the other 108 *Stevia* species studied not only confirms the negative results obtained after analytical tlc, but also strongly suggests that steviol glycosides, whether sweet or nonsweet, were absent from the samples studied of these species. It may be also pointed out that it was found empirically that the passage of *Stevia* 1-butanol-soluble fractions through alumina columns enabled the removal of many colored polar constituents, while allowing the elution of the *ent*-kaurene glycosides. This stage greatly facilitated effective sweet compound detection by tlc and hplc. The persistence of detectable amounts of stevioside in the *S. phlebophylla* sample, which was one of the oldest specimens available to us (1), not only attests to the stability of this compound on protracted herbarium storage, but also the validity of this type of approach to the study of this distribution of sweet *ent*-kaurene glycosides in the genus *Stevia*.

The apparent absence of sweet *ent*-kaurene glycosides in *Stevia* leaves previously recorded by us as exhibiting slight taste sensations after organoleptic evaluation (1,2), may be explained by either the presence of sweet constituents other than *ent*-kaurene glycosides in the specimens, or as a result of errors in the subjective judgement of the sweetness of these samples. Both the 1-butanol- and aqueous-soluble fractions of these slightly sweet species were analyzed by tlc, in an attempt to rule out the presence of low concentrations of rebaudiosides D and E, which are preferentially soluble in water (28). Also, our failure to detect isosteviol on the acid hydrolysis of the 1-butanol fractions of these slightly sweet species appears to preclude the existence of novel *ent*-kaurene glycosides, since such compounds would presumably also hydrolyze to isosteviol under these conditions. In view of the detection here of stevioside in a *S. phlebophylla* sample that was perceived as being no more sweet than the other slightly sweet *Stevia* leaf specimens tasted (1,2), it would seem unlikely that undetected trace amounts of *ent*-kaurene glycosides were present in the slightly sweet species other than *S. phlebophylla*.

From a chemotaxonomic standpoint, the occurrence of stevioside in both *S. rebaudiana* and *S. phlebophylla* is somewhat unexpected. These species are distantly separated, taxonomically as well as geographically. *S. rebaudiana* is restricted in its natural distribution to Paraguay, in the Cordillera of Amambay in the northeastern part of the country, whereas *S. phlebophylla* is restricted to Mexico, in the mountains surrounding Guadalajara in the State of Jalisco. The major taxonomic differences between these two species lie in the habit of the plants, the arrangement of capitula, and the type of achenes. *S. rebaudiana* is a rhizomatous perennial herb, with paniculately arranged capitula, and multiaristate achenes, while *S. phlebophylla* is a large shrub with corymbously arranged capitula, and non-aristate achenes (29,30).

Grashoff (30) recognized 3 subgeneric groupings of the North American *Stevia* (Colombo-Panamanian border to southern United States), with taxonomic status of Series, on the basis of habit and arrangement of capitula. These three Series are *Podocephalae*, *Corymbosae*, and *Fruticosae*. Members of the Series *Fruticosae* consists of shrubs, within which *S. phlebophylla* belongs. According to Robinson (31), three subgeneric groupings with the taxonomic status of Sections may be recognized for the Peruvian, and other South American *Stevia*, namely *Eustevia*, *Multiaristatae* and *Breviaristatae*, all of which are herbaceous in habit. Members of the Section *Multiaristatae*, within which *S. rebaudiana* belongs, are characterized by the possession of achenes with a multiaristate pappus (20-30 awns per achene). It is interesting to note that achenes with a multiaristate pappus are completely absent in North American *Stevia* species (30). Grashoff (30) has speculated that the multiaristate members of the genus appear to represent the more primitive and ancestral forms, whereas the North American species represent a derived condition, through the total or partial loss of awns. Hard and substantial evidence, however, is needed to support Grashoff's assertions.

Rebaudiosides D and E were isolated from an aqueous-soluble extract of *S. rebaudiana* leaves⁷, utilizing repeated gradient elution column chromatography over silica gel⁸, with mixtures of chloroform-methanol-water in the proportions of 45:15:2 to 9:9:2. Isolated rebaudiosides D and E were recrystallized from water-methanol and exhibited mp and optical rotation data consistent with literature values (34).

MICROEXTRACTION PROCEDURE. - Powdered *Stevia* leaves (ca. 50-100 mg per sample) were macerated in 3 X 7 ml portions of methanol-water (4:1), with each maceration stage lasting 48 hr. Macerates were combined, filtered, with solvent being removed under reduced pressure at 55°. Each residue was dissolved in water (10 ml) and defatted with 3 X 3 ml ethyl acetate. Aqueous layers were partitioned with 1-butanol (3 X 5 ml), with the combined organic phases being washed with water and evaporated to dryness. The ethyl acetate and aqueous layers of each sample analyzed were also dried and stored.

1-Butanol extracts were passed through Pasteur pipettes plugged with cotton and packed with aluminum oxide⁹ (2 g, neutral activity I), eluted with 20 ml methanol-water (1:1). Samples were prepared for preliminary tlc by removing solvent under reduced pressure.

ANALYTICAL THIN-LAYER CHROMATOGRAPHY. - Portions (ca. 10-20 μ l) of ca. 1% w/v solutions in methanol of the 1-butanol extracts of each *Stevia* leaf sample (stored and fresh were subjected to analytical tlc on silica gel GHLF (250 μ m, 20 X 20 cm) in solvents S₁ (ethyl acetate-ethanol-water, 130:27:20), S₂ (chloroform-methanol-water, 6:3:1, lower layer), and S₃, ethyl acetate-acetic acid-water (8:3:2). Plates were visualized after development using the spray reagents R₁, naphthoresorcinol-sulfuric acid¹⁰, R₂, acetic anhydride-sulfuric acid¹⁰, and R₃, 60% w/v sulfuric acid. TLC plates were heated at 110° for 10 min after spraying, and were examined in both daylight (D) and long-wave ultraviolet light (UVL).

Preliminary identifications of sweet *ent*-kaurene glycosides present in the *Stevia* leaf 1-butanol extracts were made by comparison with the following analytical TLC data obtained for the sweet *S. rebaudiana* *ent*-kaurene glycosides of known structure: dulcoside A, R_F : S_1 0.28, S_2 0.24, S_3 0.46; colors with acid sprays: R_1 red-purple (D), purple (UVL), R_2 yellow-gray (D), pink (UVL), R_3 green-brown (D), pink (UVL). Rebaudioside A, R_F : S_1 0.13, S_2 0.13, S_3 0.27; colors with acid sprays: R_1 pink (D), purple (UVL), R_2 gray (D), pink (UVL), R_3 gray (D), purple (UVL). Rebaudioside B, R_F : S_1 0.22, S_2 0.22, S_3 0.47; colors with acid sprays: R_1 pink (D), purple (UVL), R_2 gray (D), pink (UVL), R_3 gray (D), purple (UVL). Rebaudioside C, R_F : S_1 0.17, S_2 0.14, S_3 0.38; colors with acid sprays: R_1 red-purple (D), purple (UVL), R_2 yellow-gray (D), pink (UVL), R_3 green-brown (D), pink (UVL). Rebaudioside D, R_F : S_1 0.05, S_2 0.02, S_3 0.13; colors with acid sprays, R_1 pink (D), purple (UVL), R_2 gray (D), purple (UVL), R_3 gray (D), purple (UVL). Rebaudioside E, R_F : S_1 0.06, S_2 0.03, S_3 0.16; colors with acid sprays, R_1 pink (D), purple (UVL), R_2 gray (D), yellow (UVL), R_3 gray (D), purple (UVL). Steviolbioside, R_F : S_1 0.32, S_2 0.31, S_3 0.58; colors with acid sprays: R_1 pink (D), purple (UVL), R_2 gray (D), pink (UVL), R_3 gray (D), purple (UVL). Stevioside, R_F : S_1 0.20, S_2 0.18, S_3 0.38; colors with acid sprays: R_1 pink (D), purple (UVL), R_2 gray (D), pink (UVL), R_3 gray (D), purple (UVL). The aqueous leaf extracts of *Stevia* specimens perceived as possessing a slight degree of sweetness in preliminary organoleptic studies (1,2) were also subjected to an initial TLC examination in this manner.

ANALYTICAL HIGH-PRESSURE LIQUID CHROMATOGRAPHY. - The identities of *ent*-kaurene glycosides in *Stevia* leaf extracts were further checked by hplc under the following conditions: column, Dupont Zorbax NH_2 (25 cm X 4.6 mm i.d.); detection, 210 nm; eluant, acetonitrile-water (21:4, adjusted to pH 5 with H_3PO_4); flow rate, 2ml/min; pressure, 176 bar; temperature, ambient; chart speed, 10 mm/min. Samples were applied to the column in 20 μ l aliquots (ca. 0.1 μ g/ μ l), and retention times

(R_T min) were compared with the following reference solutes: steviolbioside, 4.7; dulcoside A, 7.9; rebaudioside B, 8.9; stevioside, 12.9; rebaudioside C, 16.6; rebaudioside A, 25.3. Using this isocratic elution procedure, rebaudiosides E and D were eluted, in turn, as flat peaks with retention times in excess of 1 hr.

CONVERSION OF SWEET STEVIOL GLYCOSIDES TO ISOSTEVIOL METHYL ESTER. - Crude stevioside (23 g) was prepared from *S. rebaudiana* leaves (250 g), by methanol precipitation of a 1-butanol extract (28). Hydrolysis of this material was conducted with 20% w/v H_2SO_4 (27) (750 ml) on a steam bath for 5 hr. The reaction mixture was cooled and extracted with chloroform (5 X 300 ml), and the pooled chloroform layers washed with water and evaporated *in vacuo* to yield a residue. This residue, when chromatographed over 200 g of silica gel⁸ by elution with chloroform, afforded isosteviol, after removal of solvent and crystallization from methanol, mp 221-223, $[\alpha]_D^{25} +78.1^\circ$ (c 0.28, $CHCl_3$) [lit. mp. 223°, $[\alpha]_D^{24} -69.3^\circ$ (c 0.075, 95% EtOH)] (36); uv, λ_{max} 202 nm (log ϵ) 3.17; ir, ν_{max} (KBr) 3400-2850 br, 1735, 1688, 1450, 1255, 1181, 1110, 1088, 980, 845 and 805 cm^{-1} ; pmr, (CDCl₃, 60 MHz) δ 0.79, 0.98 (6H, two s, 10-, 13-CH₃), 1.25 (3H, s, 18-CH₃), 1.50-2.84 (18H, m, methylene-CH₂) and 11.3 ppm (1H, br s, CO₂H); ms, m/z 318 (M^+ , 49%), 300 (35), 275 (27), 274 (26), 273 (21), 272 (21), 259 (20), 203 (22), 165 (35), 152 (49), 121 (57), 109 (67), 107 (56), 95 (52), 93 (55), 91 (47), 81 (77), 79 (55), 67 (51), 55 (80) and 41 (100); elemental formula determination¹¹, found C 75.37%, H 9.37%, O 15.11%, $C_{20}H_{30}O_3$ requires C 75.47%, H 9.43%, O 15.09%; R_F , toluene-acetone-acetic acid (200:55:0.7) (S_4) 0.55; isopropyl ether-acetone-acetic acid (160:45:0.5) (S_5) 0.69; color with acid spray, R_3 gray-brown (D), purple (UVL).

Isosteviol was methylated by treatment overnight with diazomethane¹² and subjected to gc/ms under the following conditions: column, glass (2 m X 3 mm); stationary phase, 0.3% OV-101 on Gas Chrom Q (80-100 mesh); column temperature,

200-360°, programmed 4°/min; He flow rate, 18 ml/min; interface temperature, 290°; spectra were recorded with 6 sec between scans. Isosteviol methyl ester exhibited the following data: R_T 6.8 min; MS m/z 332 (M^+ , 70%), 310 (15), 300 (66), 289 (17), 273 (100), 272 (42), 259 (24), 203 (19), 152 (24), 123 (31), 121 (67), 109 (58), 107 (52), 101 (35), 95 (38), 81 (51), 79 (49), 55 (61) and 41 (64).

Samples (ca. 5 mg) of dulcoside A, rebaudiosides A-E and steviolbioside were refluxed at 100° with 10 ml 20% w/v H_2SO_4 for 2 hr. On cooling, the reaction mixtures were extracted with chloroform (3 X 15 ml). The combined chloroform layers were washed with 20 ml water. On removal of solvent and analytical tlc of 10-20 μ g portions in S_4 and S_5 , isosteviol was found to be a major product for each starting compound. Identity was confirmed in each case by the detection of isosteviol methyl ester by gc/ms after methylation with diazomethane¹².

The 1-butanol fraction (ca. 2-10 mg) of each *Stevia* leaf herbarium specimen studied was also subjected to the above hydrolysis, work-up and analytical tlc procedure, in order to detect the presence of isosteviol as a hydrolytic product from any steviol (13-hydroxy-*ent*-kaurenoic acid) glycosides originally present. Isosteviol methyl ester, produced in each case by methylation of the acid-hydrolyzed *S. rebaudiana* and *S. phlebophylla* leaf herbarium samples, was indistinguishable by gc/ms from the authentic sample described above.

ACKNOWLEDGMENTS

This study was supported by contract NIH-N01-DE-02425 with the National Institute of Dental Research, Bethesda, MD. Dr. N.R. Farnsworth, Director, Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, is to be particularly thanked for his encouragement of this screening study. We are very grateful to Dr. L.I. Nevling, Jr., Director, and Dr. W.C. Burger, Chairman, Botany Department, Field Museum of Natural History, Chicago, for permission to use the *Stevia* herbarium collection for the present study. We also wish to acknowledge Mr. F. Suzuki and Dr. R.H. Dobberstein, Stevia, Inc., Arlington Heights, IL, for a generous supply of *S. rebaudiana* leaves, cultivated in California, and Drs. R. Ferreyra and C. Vargas (Peru) and Drs. A. Gómez-Pompa and M. Souza (Mexico) for assistance during the field study of *Stevia*. C.M.C. thanks the National Autonomous University of Mexico (UNAM) for financial assistance during this work.

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FOOTNOTES

1. Part IV in the series "Potential Sweetening Agents of Plant Origin". For the previous article in this series, see reference 1.
2. Contribution No. 22 from the Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago.
3. Research Associate, Field Museum of Natural History, Chicago, Illinois 60605.
4. Present address: College of Pharmacy and Health Sciences, Northeast Louisiana University, Monroe, Louisiana 71209.
5. Department of Nutrition and Medical Dietetics, University of Illinois at Chicago, Chicago, Illinois 60612.
6. Melting points were measured with a Kofler apparatus and are uncorrected. Optical rotations were determined with a Perkin-Elmer 241 polarimeter. A Beckman DB-G grating spectrophotometer was used to obtain the uv spectrum. The ir spectrum was obtained with a Nicolet MX-1 FT-IR spectrophotometer, and the pmr spectrum was recorded in deuteriochloroform, using TMS as internal standard, on a Varian T-60A instrument (60 MHz), with a Nicolet TT-7 Fourier Transform attachment. The low-resolution solid probe mass spectrum was obtained on a Varian MAT 112S instrument, operating at 70 eV. Analytical hplc was conducted using a Beckman Model 324 Gradient Liquid Chromatograph, equipped with a Model 421 Microprocessor Solvent System Controller and a Model 210 sample injection valve, and a Perkin-Elmer LC-85 Spectrophotometric Detector and LC Autocontrol. Combined gc/ms (70 eV) was performed on the Varian MAT 112S mass spectrometer, linked to a Varian 1440 model gas chromatograph and a Varian 166 data system.
7. Kindly provided by Stevia Inc., Arlington Heights, IL.
8. Merck, Darmstadt, W. Germany.
9. Woelm, Eschwenge, W. Germany.
10. Constituted according to Stähl (35).
11. The elemental analysis was determined by Micro-Tech Laboratories, Inc., Skokie, IL.
12. An ethereal solution of diazomethane was prepared using a Diazald Kit, Aldrich Chemical Compant, Inc., Milwaukee, WI.

Missouri
Botanical
Garden



26 December 1984

Dr. Robert M. King
Department of Botany
Smithsonian Institution
Washington, D.C. 20560

Dear Dr. King,

I am presently compiling data on Bolivian plant collectors, for an index consisting principally of information on itineraries; number of collections made, where they are deposited; and any special notes on the type of collections, e. g. ethnobotanical collections, wood collections, principal groups, etc. I have found the most useful source of data to be photocopies of field books (catalogues). If you would be willing, I would very much appreciate a copy of the entries dealing with the collections you made in Bolivia. If this is not acceptable, a summary of the localities visited, dates and the number of collections made at each locality would be adequate for my present purposes.

Your help with this compilation project would be greatly appreciated. I look forward to hearing from you in the near future.

Sincerely yours,

James C. Solomon
Assistant Curator

Proyecto Flora Boliviana
Casilla 20206
La Paz, Bolivia
~~79-12-84~~ TEL: 79-38-47

P.O. Box 299
St. Louis, Missouri 63166
314 577 5100

April 20, 1985

Dr. James C. Solomon
Proyecto Flora Boliviana
Casilla 20206
La Paz Bolivia

Dear Dr. Solomon:

Enclosed please find the copies that you requested. Please forgive the long delay, But I have just returned from Europe.

Regarding these collections:

First set is USNH

Second set is MEC

Third set is Kew

There is no set in Bolivia, at the time they had no place for it.

We did make ca 5 sets when possible.

Most specimens are vouchers for cytological or biochemical studies.

Many papers have already been published.

Hope that this helps.

Sincerely,

Robert M. King
Department of Botany

Missouri
Botanical
Garden



12 May 1985

Dr. Robert M. King
Department of Botany
Smithsonian Institution
Washington, D.C., 20560

Dear Bob,

Thanks very much for the photocopy of your field book
covering your Bolivian collections. They will help me a great
deal with my index.

If I can be of any help to you from here, please let me

Digitized by Hunt Institute for Botanical Documentation

Sincerely yours,

James C. Solomon
Assistant Curator

Proyecto Flora Boliviana
Casilla 20206
La Paz, Bolivia
~~79-12-04~~ TEL: 79-38-97

P.O. Box 299
St. Louis, Missouri 63166
314 577 5100

June 18, 1985

Dr. Jame C. Solomon
Proyecto Flora Boliviana
Casilla 20206
La Paz, Bolivia

Dear Jim,

I am glad to learn that my field notes in Bolivia may be of some use to you.

I have wondered if it would be possible for us to do some collecting together. We might make a good team. I would like to collect toward Santa Cruz. I would collect Compositae and you could collect anything else. I don't have much money but I could probably stay about a month.

Please think this over and let me know.

Sincerely,

Robert M. King

Department of Botany

P. S. Did you hear the good news about Peter receiving the MacArthur Award for \$240,000 ?

November 5, 1985

Dr. James C. Solomon
Proyecto Flora Boliviana
Casilla 20206
La Paz, Bolivia

Dear Jim,

Thanks so much for your letter. I do understand the mail problems that exist outside of the US. Sometimes I wonder that any mail gets thru!

I would really like to make a trip in Bolivia with you in February of 1986. I am very interested in getting down into Santa Cruz and perhaps further south if time permits. I would be mainly interested in the mountains and not in the extreme lowland. Composites are not very good in the lowlands as I am sure you already know.

As I have suggested, I would collect Composites plus chemical material and buds for cytological study and you could collect whatever you want. We would each keep our own notebooks under our own collecting numbers (mine King & Solomon and yours as Solomon & King). I assume that we could share the costs of the jeep. How do you dry your plants in the field? I usually preserve mine and dry them here in the US. I will be sending everything of mine back to the US in the embassy diplomatic pouch. I could do the same for your plant as well if you like. It would cost nothing and is very safe. I would simply have to transship your thing to St Louis. What about boxes? Can you get them or should I send some to the Embassy?

I like to make many stops along the road to get comps that I see and then move along for more. Would that fit in with your collecting? I have covered a number of things but I am sure there are others. Let me have your comments.

Sincerely,

Robert M. King
Department of Botany

November 24, 1986

Dr. James C. Solomon
Proyecto Flora Boliviana
Casilla 20206
La Paz
BOLIVIA

Dear Jim,

It was good to speak with you the other day. I am very eager to make a trip to Bolivia with you. The month of February would be best for me as we mentioned. I will make my plane reservations to arrive in Bolivia on the first of February or 1st January. I will write or call you when I know exactly. As I mentioned on the phone I will need to rest for a few days when I get there to get used to the altitude.

I am very interested in getting down to Santa Cruz and perhaps even further south if time permits. I am mainly interested in the mountains and not in the extreme lowland. Composites are not very interesting in the lowland as you know.

As I have suggested, I would collect Composites plus chemical material and buds for cytological study and you would collect whatever you want. We would each keep our own notebooks under our own collecting numbers (mine as King & Solomon and yours as Solomon & King). That's just a suggestion for you. As we mentioned on the phone I would pay for half of the gas. I would pay for my own food and lodging. Should I bring a sleeping bag? I would rather not camp if I can help it. It just takes so much time. I will bring the travellers cheeks, greenbacks, and my bank book as you suggest

I will be sending my vouchers back to the US via the diplomatic pouch. We could send your material the same way except that it would have to come to the Smithsonian first and then to St. Louis. Let me know how many cartoons. They have to be regular mail size. Should I bring some cartoons? plastic bags? Let me know.

I like to make many stops along the road to get comps that I see and then move along for more. I hope that would fit your style of collecting as least for a month. I have covered a number of things but I am sure that there are others.

Sincerely,

Robert M. King

Department of Botany

March 5, 1987

Dr. and Mrs. James C. Solomon
Proyecto Flora Boliana
Casilla 20206
La Paz
BOLIVIA

Dear Jim and Andrea,

Please forgive the long delay in writing to you but there have been so many things to do. It was very nice of you to have me stay in your home while I was in La Paz. I do appreciate it.

Several weeks ago I sent you the Bolivian money that I had left over. I do not expect that you got it. I sent it registered return receipt.

I have learned that Mike Nee is not going to return to New York until May. Did he say anything about my plants or did he give them to you.

Sincerely,

Robert M. King

Department of Botany

April 22, 1987

Dr. James C. Solomon
Casilla 20206
La Paz
BOLIVIA

Dear Jim,

Thanks so much for your letter. I am pleased to learn that you did receive the Bolivian money that I sent to you. I will deposit the check today.. I wondered if the money would get thru as I had some things stolen from my luggage.

I am eager to hear the tapes that Gaston has recorded for me. It may be best to send them in the pouch.

I am very pleased to learn that you are sending material to Prof. Bohlmann. He has only told me that he has received the material but I am very sure that he is pleased. I am going to call Peter and tell him what a good job that you are doing.

Your return to La Paz sounds a bit like a nightmare.

Please give my best to Andrea. Thanks again.

Sincerely,

RM
Robert M. King

P. S. My plants have arrived and I will send you the labels after I get back from a trip to Costa Rica.

June 12, 1987

Dr. James C. Solomon
Proyecto Flora Boliviana
Casilla 20206
La Paz
Bolivia

Dear Jim,

At long last I have the labels for my collections way back when. As you will note there is nothing wonderful there except the one Stevia without a species name. If I do anything more with it I will let you know.

I hope that everything is going well for you. Bohlmann seems very pleased with what you are sending him.

Best regards to you and your wife.

Sincerely,

Robert M. King
Department of Botany

August 14, 1987

Dr. James C. Soloman
Projecto Flora Boliviana
Casilla 20206
La Paz
Bolivia

Dear Jim,

Just a short note to inquire of a few things. Have you been able to collect any more samples for Prof. Bohlmann? I am sure that he would like more species of Stevia. I would appreciate it if you continued to let me know what species you send to him.

Did you ever get the tapes of the Bolivian music for me? Incidentally, the book on the Eupatorieae will be out on October first'

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Most sincerely,

Bob

Robert M. King
Department of Botany

August 11, 1988

Dr. James C. Solomon
Casilla 20206
La Paz
BOLIVIA

Dear Jim,

Thanks so much for sending the tapes of Bolivian music. I have enjoyed them already.

Regarding the issues of Phytologia, they were intended for the Herbario or for you. In any event please keep them.

Many thanks for collecting material for Prof. Bohlmann. I was pleased to see the several papers in Phytchemistry that were based on your material.

Sincerely,

Robert M. King
Department of Botany



The Ohio State University

Department of Botany

1735 Neil Avenue
Columbus, Ohio 43210

Phone 614 422-8952

Sept 17, 1983

Dr. Robert M. King
Department of Botany
Smithsonian Institution
Washington, D.C. 20560

Dear Dr. King,

I am sorry you were not at the Grand Forks meetings, I was looking forward to meeting you again. Dr. Robinson made an effective case for the reality and stability of the characters used as generic characters in the Eupatorieae.

I would like any of your available reprints. I intend to pursue synantherology as a career, and am sure your reprints will be a valuable supplement to the upcoming Eupatorieae treatment. I would appreciate any you could send.

Sincerely,

A handwritten signature in blue ink that reads 'David M. Spooner'.

David M. Spooner
Graduate Teaching Associate

February 9, 1988

Dr. Brian L. Stannard
Royal Botanic Gardens
Kew, Richmond
Surrey TW9 3AB
England

Dear Dr. Stannard:

Thanks for your letter of 7 January.

I am pleased to learn of your project with Ray Harley. I could send you xerox copies of my note book covering Pico das Almas if that would be acceptable to you. I note that the Florula of Mucuge did not include the new genus and species *Bishopalea erecta* H. Robinson. I believe that I sent an isotype to Kew.

I would very much appreciate a copy of the Florula of Pico das Almas when published. Also, could you send me a copy of the Mucuge paper?

Thank you,

Sincerely,

Robert M. King

Department of Botany

March 8, 1988

Dr. Brian L. Stannard
Royal Botanic Gardens
Kew, Richmond
Surrey, TW93AB
England

Dear Dr. Stannard:

Many thanks for sending me the copy of the Florula of Mucuge.

Enclosed please find xerox copy of my collections on Pico das Almas. I believe that you should have collections of most as I sent the third set of my collections to Kew. I would also be pleased to receive a copy of the Pico das Almas publication.

Sincerely,

Robert M. King
Department of Botany

357-2545

August 19, 1982

Dr. Frans A. Stafleu, Editor
Taxon, IAPT
Rm. 19.02 Tweede Transitorium
Uithof, Utrecht, Netherlands

Dear Dr. Stafleu:

Enclosed please find photographs of the following botanists:

E. Kaun, A. P. Decandolle, N. Harriman, R. Jansen, O. Huber,
D. Kaun, J. Steyermark, C. Jeffrey, L. E. Bishop, S. Mori,
O. Tovar, D. Keil, G. Nason, D. Rindos, S. McDaniel, E.
Schilling, B. Nordenstam, V. Badillo.

I hope that this series will be a welcome addition to your
collections.

Sincerely,

Robert M. King
Department of Botany

RMK:cap
Encls.

June 10, 1983

Dr. Julian A. Steyermark
Instituto Botanico
Apartado 2156
Caracas, Venezuela

Dear Julian,

I hope that this letter finds you in good health etc. It was nice that you could spend some time in Washington when you were last here in the states. There are some new spots to visit when you come back.

The reason that I am writing is to request a reprint of one of your papers, the Flora del Auyan-tepui. In fact, could you spare two copies? The second copy would be for my good friend, Earl Bishop. If you knew him you would give him a copy. Thanks in advance.

Sincerely yours,

Robert M. King
Department of Botany

357-2545

August 4, 1982

Dr. Michael Stieber
Hunt Institute for Carnegie-Mellon Univ.
Pittsburgh, PA 15213

Dear Dr. Stieber:

Enclosed please find photographs of the following botanists:

E. Kaun, A. P. Decandolle, N. Harriman, R. Jansen, O. Huber,
D. Kaun, J. Steyermark, C. Jeffrey, L. E. Bishop, S. Mori,
O. Tovar, D. Keil, G. Nesom, D. Rindos, S. McDaniel,
E. Schilling, B. Nordenstam, V. Badillo.

I hope that this series will be a welcome addition to your
collections.

Sincerely,

Robert M. King
Department of Botany

RMK:cap
Enclosures



HUNT INSTITUTE for BOTANICAL DOCUMENTATION
Carnegie-Mellon University · Pittsburgh, Pennsylvania 15213

Archives

Telephone (412) 578-2437

12 August 1982

Dr. Robert M. King
Department of Botany NHB-166
Smithsonian Institution
Washington, D.C. 20560

Dear Bob:

I received today your gift of portraits of the following
botanists:

E. Kaun, A. P. Decandolle, N. Harriman, R. Jansen, O. Huber,
D. Kaun, J. Steyermark, C. Jeffrey, L. E. Bishop, S. Mori,
O. Tovar, D. Keil, G. Nesom, D. Rindos, S. McDaniel,
E. Schilling, B. Nordenstam, V. Badillo.

Thank you on behalf of the Hunt Institute.

Unfortunately I have not been able to locate a portrait of
Christian Friedrich Lessing, "the Composite man." I checked Tax.
Lit. II for a lead, but it gives no indication of any portrait in
existence. Admitted, this is not our final resource, but one would think
that an obituary would have had a portrait of this rather eminent man.
Have you tried the literary sources of portraiture, since Lessing's
relatives include a poet and a painter? I have not done so. I did
refer to our copies of the portrait information at Geneva, but again no
luck. If anything turns up as I proceed on the Catalogue of Portraits,
I shall let you know.

Sincerely,

MTS:km

Michael T. Stieber
Archivist

357-2545

June 10, 1981

Mr. Oliveira Tangari,
Director
Faculdade Federal de
Odontologia de Diamantina
Rua da Gloria, 187
Minas Gerais, Brasil

Dear Dr. Tangari:

I would like to take this opportunity to thank you
for your help while I was in Diamantina.

Very sincerely,

Robert M. King
Department of Botany

357-2545

January 27, 1982

Dr. Oscar Tovar
Herbario San Marcos
Museo Historia Natural
Casilla 11434
Avenida Arenales 1256
Lima 14, Peru

Estimado Doctor Tovar:

Fue un placer visitarle y hablar con usted durante mi viaje a Peru. Le doy las gracias por su ayuda.

Estoy planeando volver a Peru el proximo ano y espero volver a verle.

Carmen le envia saludos.

Sinceramente,

Robert M. King
Departamento de Botanica

PROPOSAL NO. BSR-8313008	INSTITUTION UNIV OF TEXAS-AUSTIN	PLEASE RETURN BY
PRINCIPAL INVESTIGATOR TURNER	B L	NSF PROGRAM SYSTEMATIC BIOLOGY PROGRAM
TITLE DOCTORAL DISSERTATION RESEARCH IN SYSTEMATIC BIOLOGY		

COMMENTS (QUALITY OF THE PROPOSED RESEARCH, RECENT RESEARCH ACHIEVEMENTS OF THE PRINCIPAL INVESTIGATOR(S), ETC.)
CONTINUE ON ADDITIONAL SHEET(S) AS NECESSARY.

I have every reason to expect good results from this study. The group has been set up so well that a study like this can hardly fail. Additional chromosome counts and chemical studies should be very instructive. With regard to chemical samples, we suspect that very large samples will be necessary because climatic factors will probably make for a lesser concentration of the metabolites.

I don't believe that enough money has been requested for travel to Costa Rica. \$10.00 per diem is not enough! The shipping of large amounts of chemical samples has not been budgeted.

Additional chromosome numbers have been reported in King et al, 1976.

Additional synonymy as follows:

Eupatorium altiscandens McVaugh, Contr. Univ. Mich. Herb. 9:390. 1972. =
Neomirandea araliifolia (Less.) K. & R.

Eupatorium oreophilum L. Williams, Fieldiana Bot. 36:96(97) 1975. =
Neomirandea biflora K. & R.

Eupatorium orogenes L. Williams, Fieldiana Bot. 36:97(98) 1975. =
Neomirandea parasitica (Klatt) K. & R.

OVERALL RATING: ☐ EXCELLENT ☒ VERY GOOD ☐ GOOD ☐ FAIR ☐ POOR

Verbatim but anonymous copies of reviews will be sent only to the principal investigator/project director. Subject to this NSF policy and applicable laws, including the Freedom of Information Act, 5 USC 552 and formal requests from Chairpersons of Congressional committees having responsibility for NSF, reviewers' comments will be given maximum protection from disclosure.

REVIEWER'S SIGNATURE

Robert M. King

OTHER SUGGESTED REVIEWERS (OPTIONAL)

REVIEWER'S NAME (TYPED)

REVIEWER'S COPY

RUN DATE: 05/05/83

357-2545

September 1, 1981

Dr. Warwick Vincent
Freshwater Section, DSR
Tuwhasetoa St.
Taupo, New Zealand

Dear Warwick:

Just a short note to let you know that I was very pleased to meet you during the Botanical Congress in Sydney.

I hope to see you again when I visit New Zealand.

With kind regards.

Sincerely yours,

Robert M. King
Department of Botany

Oct., 6th, 1982

Dr. R. M. King
Department of Botany,
Smithsonian Institution,
Washington, D.C. 20560
U.S.A.

Dear Dr. King:

I am writing to you inquiring about the possibility
of your sending me some living achenia of eupatorias (Eupatorium
sensu strict) of eastern North America. As stated in the article,

I truly am interested in extending my research beyond
a regional basis and in comparison of cytological, ecological
and geographical data between Asian and eastern North American
species.

your cooperation will be highly appreciated.

Kobe University

Very sincerely yours

K. Watanabe
(Associate Professor of Biology)

Nov 19, 1982

Dr K Watanabe
Department of Biology
Kobe University
Kobe Japan

Dear Dr Watanabe

Enclosed are some seed of
Eupatorium that you requested.

I hope that they will germinate.

Please let me know if you
receive them. I am not sure of
your address as I was on the
envelope that I gave a friend
for the stamps

Sincerely
Robert M King

Nov. 25th, 1982

Dr. R. M. King

National Museum of Natural History,
Smithsonian Institution,
Washington, D.C. 20560

Dear Doctor King:

Thank you for your letter dated Nov. 19, 1982.

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I am most grateful for seeds of Eupatorium you enclosed.

I start to incubate them, but I am afraid that most of seeds of E. fistulosum are empty. I suppose that they were collected before full-ripening. My correct address is

Dr. KUNIAKI WATANABE

Biological Institute,
Faculty of General Education,
Kobe University, Kobe, 657, Japan

I would like to thank you once again for
your cooperation.

Sincerely yours

Kuniaki Watanabe

357-2546

August 25, 1983

Dr. K. Watanabe
Department of Botany
Kobe University
Kobe, Japan

Dear Dr. Watanabe:

Last year I sent you some seed of *Eupatorium* species. I believe that some of the seed that I sent to you did not contain the embryos that you needed. If you need more, please let me know. Many species of *Eupatorium* are now coming into flower. I would be interested in any results of your research.

Sincerely yours,

Robert M. King
Department of Botany

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Dear Dr. King:

April 25th 1984

I could make the karyotype analysis on E. fistulosum
supplied kindly from you. This karyotype of fistulosum
is clearly different from those of Asian Eupatorium species.

^{See a copy of karyotypes}
(Seven pairs of meta-submetacentric chromosomes in fistulosum
four pairs of meta-submetacentric chromosomes in Asian species.)

I am preparing a paper including these results.

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I thank you for your kind cooperation.

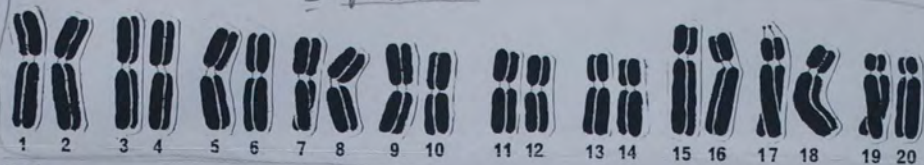
I enclose the sketch of karyotype of E. fistulosum and
some Asian representatives.

With best wishes,

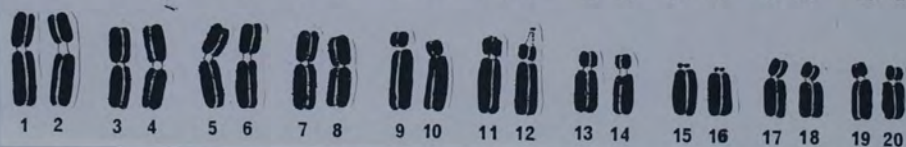
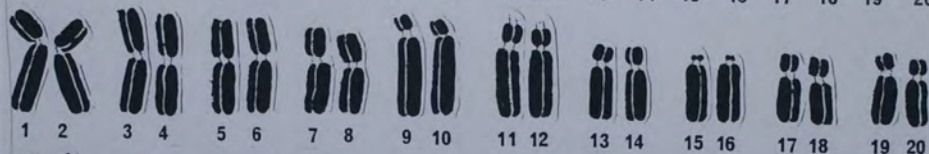
Sincerely yours

Kuniaki Watanabe

E. fistulosum



N. A. eupatorium



Asian eupatoria

357 2546

May 1, 1984

Dr. K. Watanabe
Biological Institute
Faculty of General Education
Kobe University
Kobe 657
Japan

Dear Dr. Watanabe:

Thanks so much for the reprint and the karotype drawing. I am glad that you are making so much progress. I wonder if you would be interested in working with some material that I have collected in South America. We could make a joint publication.

Enclosed are some packets of seed that I collected for you. Some I did not make vouchers, so I don't know the names for them. If you grow them you could identify them when they flower. Let me know how they do

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Sincerely,

Robert M. King
Robert M. King

Department of Botany

May 10th 1984

Dr. R. M. King

Department of Botany,
National Museum of Natural History,
Smithsonian Institution,
Washington D.C. 20560
U.S.A

Dear Dr. King:

Many thanks for your letter dated May 1st 1984 and
achene of 3 species Eupatorium and one species Ageratina. These
are really helpful to me.

I am very interested in working with South American materials.
But, I cannot afford to study them at present because
I devote myself to engage in three projects.

After I shall complete the first stage of my Eupatorium
work, I would like to join your South American work.
I am very sorry not to help your work at present.

Best wishes

Sincerely
K. Watanabe

I should be grateful for copies of your papers on Eupatorieae.
Some of them is simply not available at my end.

357-2546

July 27, 1984

Dr. K. Watanabe
Biological Institute
Faculty of General Education
Kobe University
Kobe 657
Japan

Dear Dr. Watanabe:

Enclosed please find a packet of seed for your studies. The plant is a weed so it should easily grow. Please let me know of your results.

Sincerely,

Robert M. King
Department of Botany

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January 5, 1987

Dr. K. Watanabe
Biological Institute
Faculty of General Education
Kobe University
Kobe 657
Japan

Dear Dr. Watanabe:

I want to thank you very much for sending me a reprint of your very excellent paper on Eupatorium. In my book on the tribe Eupatorieae which will come out this year I decide to sink the genus Eupatoriadelphus under Eupatorium.

I would like to request that you send me some seed of the species of Eupatorium that grow in Japan. I need these for my biochemical studies of the tribe. Anything that you could send would be most welcome.

Robert M. King
Sincerely,

Robert M. King
Department of Botany

P.S. I wish I could get you
interested in the cytology of other
genera of the Eupatorieae.

Feb 24, 1987

Dear Dr. Watarabe

Thanks so much for the seed of
Eupatorium. We have planted these
already.

I was just in Bolivia and
looked for *Aeschynanthus* seeds
for you but no luck.
I will try another source

Sincerely

Robert M. King

March 24, 1987

Dr. Kuniaki Watanabe
Biological Institute
Faculty of General Education
Kobe University
Kobe 657, JAPAN

Dear Dr. Watanabe:

I am writing to invite you to coauthor a paper with me on the cytology of the genus Neomirandea. The genus is interesting because it appears to have two base chromosome numbers, namely 17 and 25. Not all of the species have been counted but I expect that the two bases will remain. I plan to spend 2 weeks in Costa Rica next month collecting buds, seeds, and material for chemical analysis. With this material I would hope to prepare a paper on the genus. My request to you is to ask that you make a study of some of the karyotypes. For this study I will collect viable seed of a number of the species and would send these to you. I will await your reply.

Regarding your request for seed of Austroeupatorium. The genus is usually quite weedy, but I could not find any. I have a friend in Ecuador that I hope will get some for you. In any event, I will continue to look for on my future trips to South America.

Sincerely,

Robert M. King
Robert M. King

Department of Botany

April 22, 1987

Dr. K. Watanabe
Biological Institute
Faculty of General Education
Kobe University
Kobe 657
Japan

Dear Dr. Watanabe:

Thanks for your letter of 1 April.

I am pleased to learn that you are willing to work with us on the genus Neomirandea. Your question regarding the size of the chromosomes of Neomirandea, I have been told that their chromosomes are neither large or small. In any event, they are larger than the ones that Grant had in mind. I am speaking of those with 17 chromosomes.

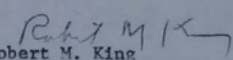
In any event I will collect seed of as many as possible for you to grow. I leave on Saturday April 25 and return in May.

I hope to see you in Berlin. My problem is money.

You know that the American dollar is not worth as much as it used to be.

With kind regards,

Sincerely,


Robert M. King

Department of Botany

May 15, 1987

Dr. K. Watanabe
Biological Institute
Faculty of General Education
Kobe University
Kobe 657
Japan

Dear Dr. Watanabe:

Please find enclosed the packet of Neomirandea seed that I collected for you in Costa Rica. I did not get as many samples as I had hoped for. There simply were not many in flower. I hope to go on another trip later.

I will be very interested in learning which ones

you are able to grow.

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Please forgive me but my
typewriter stopped working

Sincerely

Robert M. Kim

May 29, 1967

Dr. Kunisaki Watanabe
Biological Institute
Faculty of General Education
Kobe University, Kobe 657
Japan

Dear Dr. Watanabe:

Thanks for letters and the packets of Neomuranthus seed. I apologise for the misunderstanding. I sent the seed to you first with only collection as I did not know the species. I never did not plan to furnish you with any information that you might need and that I knew. I am returning the packets of seed to you with the information that you requested. Please let me know what other information you require. I only sent you the seed promptly as I don't know how long the seed is viable.

On another matter I am working on getting you seed of Austrocyathus. Please let me know what information you need so that the seed is useful to you.

Sincerely,

June 18, 1987

Dr. K. Watanabe
Biological Institute
Faculty of General Education
Kobe University
Kobe 657
Japan

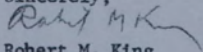
Dear Dr. Watanabe:

Thanks for your recent letter. I am glad that the seeds of *Neomirandea* arrived safely. I understand appreciate your situation especially with no assistant. It will be good if you can get the seed to germinate before your trip. I do not know how long the seed may remain viable. I will hope to send you more seed from time to time.

Enclosed are some reprints regarding *Neomirandea*. Please let me know how else I may help you.

Digitized by Hunt Institute for Botanical Documentation

Sincerely,



Robert M. King

Department of Botany

Dr. R. M. King

Department of Botany,
National Museum of Natural History,
Smithsonian Institution,
Washington, D.C. 20560, U.S.A.

June 27th 1987

Dear Dr. King:

Thank you for Four papers of Neomirandea. I tried
to test the germination of seeds and N. guerrarii (No. 9674) and
N. angularis (No. 9695) could germinate. ^(not so good seeds) Other seeds of species
^(very good seeds) N. eximia ^(N. parasitica) could not remain viable. In Neomirandea parasitica (No. 9670)
I could not find any mature seeds.

That is all my results at present.

I suppose that their seeds are not enough to mature.

Sincerely yours

Kuniaki Watanabe

Biological Institute
Faculty of General Education
Kobe University, Kobe, 657, Japan

July 10, 1987

Dr. K. Watanabe
Biological Institute
Faculty of General Education
Kobe University
Kobe 657
Japan

Dear Dr. Watanabe:

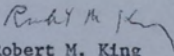
Thanks for your recent letter with the information about the germination of the Neomirandea seed. I hope to send you some more soon.

Enclosed please find some reprints on chromosome numbers. I do not recall that I sent these to you earlier. If I did, please give these to a friend.

Have a good trip to Europe.

Digitized by Hunt Institute for Botanical Documentation

Sincerely,


Robert M. King

Department of Botany

August 12, 1987

Dr. K. Watanabe
Biological Institute
Faculty of General Education
Kobe University
Kobe 657
JAPAN

Daear Dr. Watanabe:

I hope that you had a good trip to Europe and the Congress.

Enclosed is another sample of seed of *Neomirandea*. I hope that they will germinate (the seed look good). I am hoping to make another trip to Central America for more collections.

Sincerely,

Robert M. King

Department of Botany

October 15, 1987

Dr. K. Watanabe
Biological Institute
Faculty of General Education
Kobe University
Kobe 657, Japan

Dear Dr. Watanabe:

Enclosed are three more samples of Neomirandea seed that were collected for me in Panama. I do not know the species yet but I will send you the name when I see some herbarium material. I hope that you have had good luck with the material that I sent you in August. Also, I hope that you enjoyed your trip to the Congress and Europe.

Sincerely,

Robert M. King
Robert M. King

Department of Botany

Dr. R. M. King

Department of Botany,
National Museum of Natural History,
Smithsonian Institution,
Washington, D. C. 20560
U. S. A.

Nov., 19th, 1987

Dear Dr. King:

I am going to report shortly on the data of Neomirandea.
Two species could be examined. Neomirandea anglaris has
the chromosome number $2n=50$ and N. guevaru $2n=62$. In the latter
species, twelve chromosomes out of 62 are extremely smaller than
the remainings. I think that they are B-chromosomes. So
both species have the same A-chromosome number $2n=50$ which may
be pentaploid based on the $x=10$. We have to examine other
clones of N. guevaru collected from different localities.
I will do the precise karyotypic comparison after the study of
the diploid taxa (if they are present). At least both species
are not autopolyploid karyomorphologically because I can not
pick up the five set of distinct chromosomes. Now I am
(largest metacentric chromosome or satellite subterminal chromosomes)
cultivating one other N. anglaris strain, other seeds of species
could not be germinated. If you want to go on this work,
please send me "good matured seed".

Sincerely yours

Kuniaki Watanabe

Kuniaki Watanabe

Biological Institute,
Faculty of General Education,
Kobe University, Kobe, 657,
Japan

Notes

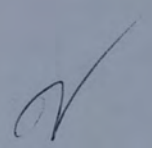
Mitotic analyses were made on seedling root tips after treatment in 0.004 mol. 8hydroxy-quinoline solution at 20°C for 2 hours. Fixation, staining and maceration were made in mixture of 1% acetic orcein(9parts) and 1 N-HCL(1 part) for 24 hours at 5°C. Subsequently they were kept in the mixture for 30 seconds at 80°C, and then squashed on the slide.

Five clones of Neomirandea anglaris(B.L.Rob.)K. & R. collected from Costa Rica, Cartago, 6200ft., May, 4th, 1987, Collection number 9695 have the chromosome number $2n=50$.

Five clones of Neomirandea guevaru R. M. King & H. Robinson collected from Costa Rica, Cartago, 4900ft., April, 28th, 1987, Collection number 9674 have the chromosome number $2n=62$.

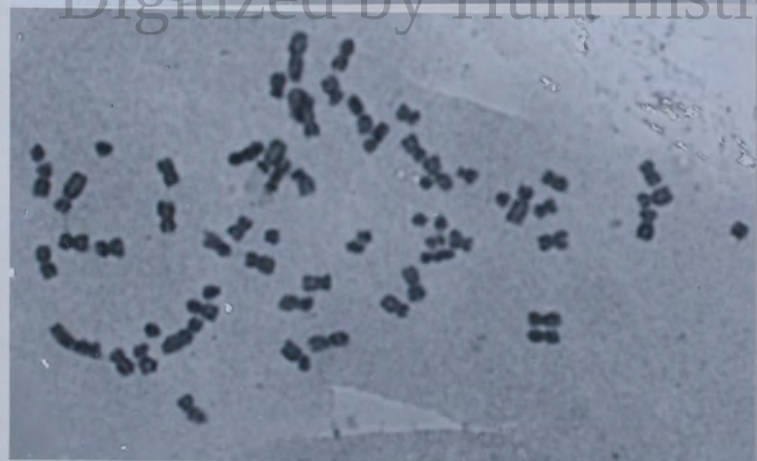
Twelve out of 62 are too small to determine their centomeric position. They seem to be B-chromosomes. If it is so, this is the first report of B-chromosome in the tribe Eupatorieae.

It is surprised that their B-chromosome number are constnt throughout the examined somatic cells and clones. Thus these plants may be produced agamosperously. We have to examine other clones collected from different localities. Both examined species may be pentaploid based on $x=10$, but not be autopolyploid karyomorphologically. 'Before precise karyotypic comparison of two species, it is necessary to examine the karyotypes of diploid taxa.



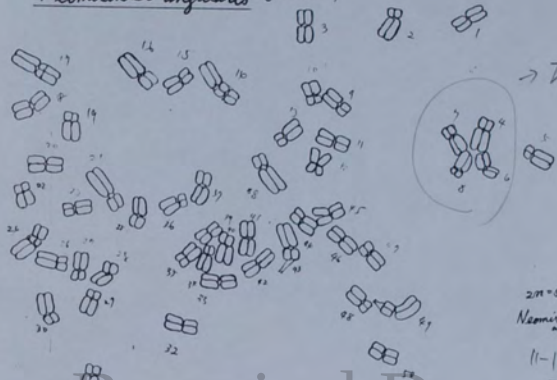


X3000



X3000

Neomirandea angularis ($2n=50$)



→ They are not 2 large chromosomes
but 4 small chromosomes

$2n=50$
Neomirandea
angularis
11-13, 13, 13, 13, 13, 13

about 3200 X

Neomirandea guerrarii
($2n=62=50L+12S$)



about 3200 X

November 23, 1957

Dr. K. Watanabe
Biological Institute
Faculty of General Education
Kobe University
Kobe 657, Japan

Dear Dr. Watanabe,

Thanks so much for your letter. I am
pleased to learn that some of the seed are growing.
I hope that you will be successful in your
studies.

I hope that you received the seed that Dr. Hillebrand
collected for us. We are growing some here in our
greenhouse.

Enclosed is some more seed of *Neomeris*
angularis collected in Costa Rica. I will give you
more information regarding these collections when I
receive it later.

Also, enclosed is some information on my
new book.

Sincerely

Robert M. King

December 8, 1987

Dr. Kuniaki Watababe
Biological Institute
Faculty of General Education
Kobe University
Kobe 657
JAPAN

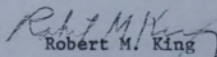
Dear Dr. Watanabe:

Thanks so much for your letter and the very interesting reports. I must say that I am very impressed with your very good work!

I hope by now that you have recieved the two other collections of *Neomirandea angularis* that I sent to you.. I am planning to visit Costa Rica and Panama during January of next year. I hope to get you more seed then. I will try my very best to get you mature seed .

Digitized by Hunt Institute for Botanical Documentation

Sincerely,


Robert M. King
Department of Botany

February 11, 1988

Dr. K. Watanabe
Biological Institute
Faculty of General Education
Kobe University
Kobe 657
Japan

Dear Dr. Watanabe,

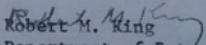
Please forgive the long delay in answering your letter. I have had some problems.

I am pleased to learn that you like our book and have ordered it. Regarding the mistake of citing *Eupatorium amabile* from Japan, I do not recall where we got the information. In fact we have no material of this species in our herbarium. Do you know where I could get or borrow this species?

I am hoping to go to Costa Rica and Panama in late March. Then I should have some seed to send you. Also, I have not forgotten about *Austroeupatorium* for you. Someday.

With kind regards,

Sincerely,


Robert M. King
Department of Botany

August 28, 1989

Dr. K. Watanabe
Biological Institute
Faculty of General Education
Kobe University
Kobe 657
Japan

Dear Dr. Watanabe:

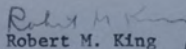
Please excuse the long delay in answering your letter. The problem was that I had no way to get you more seed of Neomirandea. This was because of financial and political reasons. I am sure that you are aware of the United States' problem with Panama's General Noriaga.

However, now the financial and political restraints no longer exist and it will be possible for me to collect material of Neomirandea in Costa Rica and Panama early next year. I hope that you are still interested in the project. Please let me know. The results that you sent earlier were very interesting and important and should be published with the photographs. But a complete study would get the same information for more species and compare the structural details of the $n = 25$ and $n = 17$ groups.

Incidentally, I have growing in our greenhouse the same species of Adenostemma that Dr. Yahara was interested in. If it flowers, I could probably provide you with seed. Also, I plan to visit South America next year and could probably get him seed of several species of Austroeupeatorium. I hope that he is still interested. I have not heard from him since May.

I eagerly await your reply.

Sincerely,


Robert M. King

Department of Botany

October 16, 1989

Dr. Kuniaki Watanabe
Biological Institute
Faculty of General Education
Kobe University
Kobe 657, Japan

Dear Dr. Watanabe:

Many thanks for your letter. Please excuse the delay in writing to you as I have just returned from a collecting trip to Mexico.

I am delighted to learn that you are still interested in the Neomirandea project. I will then plan to go to Panama and Costa Rica early next year. I will try to get as many seed of different species as possible. I may have to send you the seed with only collection numbers at first but I will send you the complete information as soon as possible. The important thing is to get the seed to germinate first, then you can study it later.

I hope that Dr. Yahara recieved my letter. I never had an answer so I do not know what to plan.

With kind regards,

Sincerely yours,

Robert M. King

Robert M. King

Department of Botany



18 Feb. 1987

Dr. R. M. King
Dept. of Botany
Smithsonian Institution
Washington, D.C. 20560

Dear Dr. King:

As of today, slides of the rest of your material were sent to Mike. As soon as he confirms the counts, you will receive the results, post haste. You will be pleased to know that Acritopappus (K8065) was determined, in late diakinesis, as $n = 9$. I will also return your empty vials shortly.

It seems that my attempts to get professional obligations completed on time continue to get side tracked, due to our workloads. It is clearly in your best interest to find someone more dependable. I have a will but, unfortunately, not a way. I would rather work on chromosomes than plan our first campus building!

I wish you and Dr. Robinson the very best on your unraveling of the mysteries of the Eupatorieae, et al.

Sincerely yours,

James F. Weedin
Science Division Chair

March 30, 1987

Mr. John L. Weiler
Hotel "Don Carlos"
Apartado 1593
San Jose
Costa Rica

Dear Mr. Weiler:

I would like to reserve a room for two people (twin beds) for the night of April 18, 1987. Would you please confirm this and indicate the cost and method of payment (cash or credit card)? We expect to spend about two weeks in Costa Rica collecting plants. Don Carlos may well remind me as I have stayed at the hotel before.

Robert M. King
Sincerely,

Robert M. King

Department of Botany

May 8, 1989

Dr. J. G. West
Herbarium Australiense
P. O. Box 1600
Canberra City
A. C. T. 2601
Australia

Dear Dr. West:

Please excuse the long delay in writing to you, but I have just returned from Europe. I want to thank you for shipping my plants to the USA. Everything arrived in good condition. As soon as I get the names from Philip Short I will send you the labels for your herbarium. Again, thanks for your help.

Sincerely,

Robert M. King
Department of Botany

June 12, 1989

Dr. J. G. West
Herbarium Australiense
P. O. Box 1600
Canberra City
A. C. T. 2601
Australia

Dear Dr. West:

Please find enclosed the labels for the specimens that I collected and left with you to dry and send to me. Thanks again for all of your help.

Sincerely yours,

Robert M. King
Department of Botany

357-2545

September 1, 1981

Dr. Arthur S. Weston
Western Australian Herbarium
Department of Agriculture
Jarrah Road
South Perth
Western Australia 6151
Australia

Dear Arthur:

I am very pleased to meet and talk with you during the Congress in Sydney. All in all, I thought that the Congress was a big success.

Enclosed is the list of determinations that we talked about. I hope that the information is there.

I have talked with the people here and it will probably be possible for me to visit and collect in Perth next August. I very much appreciate your invitation.

With kind regards.

Sincerely yours,

Robert M. King
Department of Botany

Enclosure

357-2545

December 7, 1981

Dr. Arthur S. Weston
W. Australian Herbarium
Dept. of Agriculture
Jarrah Road
S. Perth
W. Australia 6151
Australia

Dear Arthur:

I am writing again to inquire about the status of my trip to Western Australia next August. I will be able to get the money, but need to begin to plan for it.

I hope that all is well with you and your family.
With kind regards,

Sincerely,

Robert M. King
Department of Botany

357-2555

December 28, 1981

Dr. Arthur Weston
13 third Avenue
Kensington
Western Australia 6151
Australia

Dear Arthur:

I'm writing another letter to your home as you might not have received the one that was sent to the University. I am most eager to accept your offer to visit Australia in August of 1982. I hope that things are still ok. Please let me know as soon as possible.

Sincerely yours,

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Robert M. King
Department of Botany

August 9, 1985

Dr. Paul G. Wilson
Western Australian Herbarium
Department of Agriculture
George Street
South Perth
Western Australia 6151, AUSTRALIA

Dear Dr. Wilson:

By way of introduction, I am working on a book dealing with the biochemistry of the Compositae. This work is being done with Prof. Dr. F. Bohlmann of Berlin (Senior author), Dr. Harold Robinson of this institution and others. Our project involves analyzing a broad sampling of the World's Compositae. To this end, we have intensively studied taxa from Europe, Asia, North America, and South America and Africa. As you can see, we have collected and analyzed Compositae in each major geographical region, except Australia. That is the reason for writing this letter.

In order to complete our survey of the Compositae, I would like to do some collecting in Western Australia. I anticipate spending 4-6 weeks in this effort there (calendar year 1986) depending on the cost. May I request your advice regarding the best time of year for collecting Compositae in Western Australia? In addition, may I ask in what ways could you or the Herbarium help me, should you desire? Of course, I can offer some authorship in the resultant publications.

I would very much appreciate receiving your comments regarding these matters.

Sincerely yours,

Robert M. King
Department of Botany

BY AIR MAIL PAR AVION

AÉROGRAMME



North Rankin A gas drilling platform, North West Shelf, 130km off Dampier Western Australia

PERIOD

COMMON

11-16

12-17

18-19

POLL

COMMON

11-16

12-17

18-19

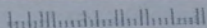
50c

AUSTRALIA



Nomad

TO Dr Robert M King
National Museum of Natural History
Smithsonian Institution *B/K6*
WASHINGTON D.C. 20560
COUNTRY OF DESTINATION U.S.A.



SENDER'S NAME AND ADDRESS

Western Australian Herbarium
DEPARTMENT OF AGRICULTURE

George Street, South Perth, Western Australia 6151

POSTCODE

TO OPEN SLIT HERE AND THIS LINE

FOLD SIDE FLAPS FIRST

FOLD SIDE FLAPS FIRST

Western Australian Herbarium
DEPARTMENT OF AGRICULTURE
George Street, South Perth, Western Australia 6151

466/74
PGW:VH

10 March 1987

Dr Robert M. King
National Museum of Natural History
Smithsonian Institution
WASHINGTON D.C. 20560
U.S.A.

Dear Dr King

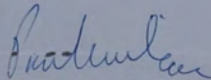
I have your letter of 24 February to Dr Green, who is on leave, with regard to your Western Australian collection of plants.

I understand from Mr Lander that your specimens were dispatched by surface mail early October. They should by now have arrived at the Smithsonian and I wonder whether they are awaiting fumigation or whatever the treatment is you give to incoming material. Before we proceed with any division of our set I would like you to confirm that there is no holdup in your institution.

The list of identifications was sent airmail to you on 21 January. Please let me know if this has still not arrived and I will then arrange with Mr Lander for a second list to be prepared.

With kind regards

Yours sincerely



P.G. Wilson
Senior Botanist

March 23, 1987

Dr. P. G. Wilson, Senior Botanist
Western Australian Herbarium
Department of Agriculture
George Street
South Perth, Western Australia 6151
AUSTRALIA

Dear Dr. Wilson

Thanks so much for your very kind letter of 10 March.

My specimens have still not arrived and it has been over 6 months. Our shipping people tell me that it should not take over 3 months for material to reach us from Australia. In view of this I do believe that the material has been lost. Would you then please divide the set that you have in Perth and send half to me via registered air mail? In addition, I still have not recieved a list of identifications. Would you please send me a copy of the list that was sent earlier?

With kind regards and thanks,

Sincerely,

Robert M. King
Department of Botany

April 21, 1987

Dr. Paul G. Wilson
Western Australian Herbarium
Department of Agriculture
George Street
South Perth
Western Australia
Australia

Dear Dr. Wilson:

Thank you for your letter of 6 April.

I am pleased to learn thaty you will now divide the set in Perth and send me half. In fact, they have alreddy arrived. Thanks so much for your help in this matter. We can now go ahead in our testing of the material. I hope to be able to send you some results soon.

Sincerely,

R. M. King
Robert M. King
Department of Botany

April 10, 1989

Dr. Paul G. Wilson
Western Australian Herbarium
Department of Agriculture
George Street
South Perth
Western Australia, Australia

Dear Dr. Wilson:

Please find enclosed two reprints on our study of Western Australia Compositae. I hope that they will be of interest to you. I have since been on another collecting trip to South Australia, Victoria and New South Wales and have collected more species for another study.

I would be pleased if you would send me reprints of your new genera when published.

With kind regards,

Digitized by Hunt Institute for Botanical Documentation

Sincerely,

Robert M. King

Department of Botany

Dr. Robert King
Department of Botany
Smithsonian Institution
Washington, D. C. 20560

Dear Dr. King:

15 Dec., 1982

I was very pleased to spend several days at the Smithsonian by the end of August. I still remember your kind reception and good suggestion to cooperate with my chinese colleagues at Institute of Botany, Academia Sinica, Peking.

About one month ago I have written a letter to introduce you to my director and the nice visit at the Smithsonian as well. Recently I wrote another letter to Dr. Y. L. Chen who is studying at Kew Botanic Garden, I guess he will intereted in the future relationships in the field of Compositae between two countries. You also can write him anytime if you would like to do that.

I will visit the Farlow Herbarium and the New York Botanical Garden pretty soon for several weeks.

I hope I will reception you in Peking in the near future and wish you a Merry Christmas and Happy New Year.

Sincerely yours,

P. C. Wu

P. C. Wu

address:

Dr. Y. L. Chen
Royal Botanic Garden, Kew
Richmond, Surrey TW9 3AB
England

May 26, 1989

Dr. Tetsukazu Yahara
Botanical Gardens, Nikko
Faculty of Science
University of Tokyo
Hanaishicho 1842
Nikko 321-14
Japan

Dera Dr. Yahara:

Thanks for your letter. As I told you on the phone, I will be glad to collaborate with you and join you on a trip to Brazil and Ecuador.

As far as I know now, no collecting permit is required for Ecuador. However, Brazil has very severe collecting laws. It will be necessary for you to apply for a permit. I strongly suggest that you do this as soon as possible. This is because it may take ~~several~~ more than one year to get the permit.

The address that you should write is:

Superintendent for International Cooperation
Conselho Nacional de Desenvolvimento
Cientifico e Tecnologico
Edificio CNPq
Av. W3 Norte Q. 507/B
CX. Postal, 11-1142
Brasilia DF-CEP. 70000

~~BBazi~~Brazil

If I can help you in any other way please let me know.

Sincerely yours,

Robert M. King

Department of Botany

357-2546

December 6, 1982

Dear Dr. Yu:

Thank you for your recent letter. I will be sending you the requested information soon.

Sincerely yours,

Robert M. King

Robert M. King
Department of Botany

Digitized by Hunt Institute for Botanical Documentation

357-2545

3 de marzo de 1981

Dra. Elsa M. Zardini
Museo de la Plata
La Plata, Argentina

Estimada Elsa:

Le doy las gracias por los libros enviados recientemente.
Los mismos son una gran aportacion para mi biblioteca.

Espero todo marche bien por alla.

Sinceramente,

Robert M. King
Departamento de Botanica

May 21, 1913

Dear Mr. King:

Just felt a note would be in order to thank you for a great come-back on my behalf.

Having not taken a class in 18 yrs. this course was really a stepping stone to see if I could get back into studying and really would want to.

You made it so enjoyable and interesting, the transition was easy. Thanks for your help. I know now that I can do it and plan to go all the way. ~~Your class was great. For me and I think~~

Mr. and Mrs. Fred L. Smith

you get the subject over in such a
way as to make it easy on and "old"
class student to get back in the grind.

Thanks again,

Gornie Jewett