V　植物学での指導報告

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本プロジェクトの植物部門（アスンシオン大学植物学部門、富山医科薬科大学薬学部附属薬用植物園）が分担すべき研究協力課題として、以下の４点がプロジェクト開始時に計画立案された。

１）パラグアイにおける民間薬および薬用植物の調査
　市場品、地方独自薬物の調査、市場品供給地の調査・見学、薬用植物および近縁種の調査（分布、生態、標本、導入、等）

２）パラグアイ民間薬の生薬学的研究
　パラフィン切片法およびスンプ法による生薬鑑定

３）パラグアイ薬用植物の栽培学的研究
　繁植方法の検討（発芽および挿木試験）栽培方法の検討（栽植時期および密度）

４）パラグアイ薬用植物の育種学的研究
　外部形態・電気泳動による地理的変異の検出

１）について

1985年５月21、22日の両日にわたり、アスンシオン大学のスタッフおよび日本からの短期派遣専門家とともに、アスンシオン市第４市場においてパラグアイ民間薬草の調査・収集を行った。調査方法は、植物名（現地名）、利用部位、利用目的などの項目についての聞き取り調査とした。両日間に得られた薬草（71品目、表１）については、写真撮影後乾燥標本とし、アスンシオン大学植物部門において整理保存することとした。その後、市場調査と植物種の同定・標本作成に関しては、日本側吉崎専門家とパラグアイ側植物部門のスタッフを中心に作業が続けられている（吉崎専門家の報告参照）。

２）について

生薬および植物の表面構造と内部構造の観察方法として、それぞれⓐスンプ法、ⓑパラフィン切片法に関する指導を行った。

ⓐ　スンプ法

スンプ法は、不透明な組織の微細な表面構造を観察するのに適した方法であり、簡便で実

用性が高い。セルロイド板の片面を酢酸アミルで溶かし、被検物を押しつけネガティブプリントを作るもので、透過光により光学顕微鏡で観察する。

1985年、C／Pとして、アスンシオン大学植物部門のネリーダ、ミルタ両氏来日時に、富山医科薬科大学薬用植物園に栽培されているマメ科Cassia属植物数種を材料として、材料植物の取扱い方とプリント方法について指導を行った。さらに、1986年C／PとしてDr.イサベル来日時に、ナス科Datura属植物を材料として前年と同様の指導を行い、光学顕微鏡による観察、写真撮影を行った。Datura属植物のうち、D. arboreaとD. suaveolensとは、葉の表面に生ずる毛のタイプが異なることが観察された。

ⓑ パラフィン切片法

パラフィン切片法は、固定組織を脱水後パラフィンブロックに包埋し、ミクロトームを用いて厚さ数ミクロンの切片を作製する方法であり、植物の内部構造観察のための永久標本作製法としては一般的な方法である。スンプ法と同様に透過型の光学顕微鏡で観察する。今回用いた方法は、固定液としてF. A. A.液（F. A. A液は、固定と同時に組織の保存液としてもすぐれている)、脱水シリーズとしてn－ブチルアルコール・シリーズおよびエチルアルコール・シリーズ、切片染色液としてデラフィールドのヘマトキシリンとした。切片作製は、回転式ミクロトームを使用した。回転式ミクロトームを用いた場合には、容易に連続切片を得ることができ植物の維管束走行などの研究に便利である。

1986年C／PとしてDr.イサベル来日時に、Datura属植物およびCapsicum属植物の葉柄を材料とし、FAA液による固定～永久切片の作製、顕微鏡写真撮影の指導を行った。さらに、スンプ法による写真とともに、現像、焼付など一連の暗室技術の指導も同時に行った。Datura属植物とCapsicum属植物については、文献記載と同様な維管束の配列が確認された。

3）について

植物の栽培化に当っては、それぞれの種について繁殖方法と栽培方法とに関する検討が必要である。しかし、本プロジェクト期間中には、その一部分についてのみ実行可能と判断される。そこで、アスンシオン大学薬用植物園設置をも考慮し当面繁殖方法の検討を行うこととした。繁殖方法は種子によるものと、植物体の一部分を用いる栄養体繁殖とに大別される。それぞれの繁殖方法に長短があることから、種子繁殖法と栄養体繁殖法の一種である挿木を行うこととし、種子については、現在アスンシオン大学植物部門のスタッフが収集、準備中である。以下挿木試験について述べる。1985年6月にアスンシオン大学において、パラグアイの薬用植物数種を用いて挿木の仕方、管理法について指導を行った。さらに、1985年および1986年C／P来日時に、富山医科薬科大学薬用植物園において進行中であった薬木43種を用いた挿木試験を協同して行い、その際、挿木の仕方、管理法および結果の調査方法について指導した。パラグア

イ薬草（薬木）の供試種および結果を表２に示した。材料入手の都合から供試本数が同一でないが、用いたすべての種について発根が認められ、栄養体による繁殖の可能性が示唆された。発根率はAmbayの10％からCangorosaの90％まで差が大きくパラグアイにおいては６～７月が秋に相当するため挿木時期が適切でなかったとも考えられる。したがって、今後供試種数を増やして、１年間を通して挿木を行い、最適な挿木時期について検討する必要があると思われる。

４）について

薬用植物を安定して供給できる様にするためには、野生植物を栽培化する（課題３））とともに、安定・多収品種の育成が必要である。そのためには、目的とする植物の様々な遺伝形質（草丈、葉の大きさ、開花時期、花色、果実の大きさ、種子数などの諸形質と生理形質）を明らかにしておくことが不可欠である。そこで、将来の品種改良のための基礎的知見を得ることを目的とし、電気泳動法による地理的変異の検出を試みることとした。

1985年C／Pとしてネリーダ、ミルタ両氏来日時に、ポリアクリルアミドを支持体とするSDS－PAGE法によるタンパクのパターン分析の指導を行った。供試材料は、富山医科薬科大学薬用植物園において栽培・調製したTrichosanthes属植物５種の根茎とした。供試５種は顕著に異なる泳動パターンを示した。そこで、一致係数によるクラスター分析を行ったところ、従来の分類と矛盾しないことが明かとなった。この泳動パターン分析の過程において、パーソナルコンピューターを用いた多変量解析法（とくに、クラスター分析）の指導も同時に行った。

C／Pの１人、ネリーダは、帰国後パラグアイ産の産地を異にするCassia属植物の種子を用い、同様の方法で地理的変異の検出を試みたが、「供試材料中に変異は認められなかった」とのことであった。この結果は、SDS－PEGE法の紹介とともに、アスンシオン大学セミナーにおいて報告された。さらに、現在彼女によって供試材料決定のためのスクリーニング作業が進行中である。

以上が各課題についてのまとめであるが、以下全体的な事柄について述べる。

現在までに、アスンシオン大学植物部門の研究室の整備もほぼ完了し（吉﨑専門家の報告参照）、本プロジェクト開始時に立案された課題を遂行するための基礎的な技術移転も一部を除き終了した。課題１）を除く他の課題については、おそらくアスンシオン大学植物部門にとって、これまでに経験のない分野であると考えられる。したがって、今後協同研究を進めて行く過程において上記移転技術に関するアフターケアと今後の研究の方向ずけが必要と思われた。

上記の観点から、1986年にアスンシオン大学植物部門の責任者であるDr.イサベルがC／Pとして来日した際に、①市場品の標本および基源植物の腊葉標本の蒐集　②アスンシオン大学薬用植物園の整備　③パラグアイ薬草栽培化のための研究　④電気泳動法による変異の検出　⑤植物採

集旅行　⑥C／P来日時の研修内容　⑦専門家派遣時期と指導内容　⑧その他の諸点について協議し、今後の研究内容について決定した。さらに、植物園整備の参考のために、日本の代表的な薬草園である武田薬品（K.K)の京都農園、京都府立植物園、京都薬科大学薬草園の見学を行った。日本における薬草栽培の現地見学として、長野県北御牧の長野県野菜花卉試験場、薬用人参、センブリの栽培地見学に同行した。

以上の様に、本プロジェクトの植物部門に関しては、研究協力の準備が一応完了した段階と考えられる。

表－1

21 de mayo de 1985

PLANTAS MEDICINALES DEL PARAGUAY (Compradas del Mercado 4)

1. Santa Lucía blanca (Refrescante)
2. Cepa caballo (Refrescante. Se machaca y toma en el agua)
3. Perdudilla (Refrescante. En agua fría)
4. Zarzaparrilla (Refrescante. En agua fría)
5. Cola de caballo (Para los riñones. En agua caliente)
6. Aguapé puruá (Para la inflamación del estómago. En agua caliente)
7. Cocú (Refrescante. En agua fría)
8. Typycha curatú (Para indigestiones)
9. Ñangapyry (Para adelgazar)
10. Capii cati (Refrescante)
11. Mbocaya-i rapo (Diuretico)
12. Batatilla (Refrescante)
13. Raíz de perejil* (Abortivo)
14. Cedrón Paraguay (Para calmar los nervios)
15. Llanten (Raíz) (Remedio para todo. Caliente)
16. Menta-í* (Para calmar los nervios)
17. Taropé (Refrescante - all part)
18. Tupasy camby (Refrescante)
19. Poleo-í (Remedio caliente en té o mate - abortivo)
20. Toronjil* (Para el corazón)
21. Ñuaty pytá
22. Urusu caty (para echar lombrices - en decocción)
23. Verbena-í (Para dolor de garganta - en decocción)
24. Cerdon capii (Para calmar los nervios - té)
25. Raíz de hinojo (Para dolor de estómago. En agua caliente)
26. Tayuya (Abortivo. Se toma en tereré o mate)
27. Ysypo mil hombre (Abortivo y refrescante. En el mate) (Aumenta virilidad)
28. Malva rapo pire (Abortivo. Decocción o té)
29. Caña brava (Abortivo. Decocción o té)
30. Yagua rova (Abortivo. Se toma té o decocción)
31. Ruibalbo (Abortivo. Se toma té o decocción)
32. Charrua caa (Para el estómago, Para despertar el apetito)
33. Chicoria (Purgante. En té o decocción)
34. Pindo rapo (Abortivo. En té o decocción)
35. Para todo pire (Uso desconocido)
36. Usuru hee (Para catarro. En mate o té)
37. Guayacan corteza (Dolor de barriga. En té o decocción)

38. Ybyrá pytá piré (Para lavar heridas o problemas de la piel. Se cocina y-lava)
39. Yateí caa (*Remedio caliente. En té o decocción*)
40. Yaguareté caa (Para el estómago. En té o decocción) (Como depurativo)
41. Colaguala (Abortivo. En té o decocción o en mate)
42. Penicilina (Para limpiar heridas. Se hierve y lavar)
43. Doradilla (Abortivo. Té o decocción)
44. Flor de mamón macho (En forma de jarabe para la tos de los niños especialmente)
45. Cangorosa (Abortivo y para úlcera. Té o decocción)
46. Malva blanca (Para eliminar catarro. Té o decocción)
47. Yuruveva
48. Ambay (Para la tos, Catarro. Té o decocción)
49. Yerba de lucero (Para el estómago. Té o decocción)
50. Pata de buey (Para los riñones. En tereré o mate)
51. Yaguá rundy (Para la tos. Té o decocción)
52. Sauco (Para inflamación del estómago. Friccionar)
53. Alcanfor del campo
54. Yateí caá (Remedio refrescante. En tereré)
55. Barba de maíz (Avatí zogüé) (Para los riñones. En té o mate)
56. Sara morotí corteza (Para diabetes. Se machaca,se hierve y se toma como agua)
57. Mecho acá raíz (Para el corazón. Se machaca y se toma con agua fría)
58. Siete sangría (Para la presión. Con agua fría o en mate)
59. Para para-í (Para los riñones, rompe piedras. Con agua fría o caliente)
60. Ysypo pere (Para el cáncer. Se machaca. En té o decocción)
61. Tapecué (Problemas de estómago. Para lavar heridas se hierve)
62. Ybahai (Para diabetes. Se hierve y se toma 2-3 veces al día o como té)
63. Siempre viva (Para el corazón y calmar los nervios: Se hierve y se toma en mate)
64. Calabacita (Para diabetes, se hieve y toma como agua o en tereré)
65. Caaré (Antihelmítico. Se hierve y se toma en ayunas)
66. Chirca melosa (Diabetes. Se hierve y se toma como agua o té)
67. Kino kino (Para dolores reumáticos y para golpes. Se machaca y se hierve)
68. Capi-una (Para el riñón. En té o decocción)
69. Agrial (Para dolor de garganta. Se hace gárgara con agua fría)
70. Curupaymí (Para el reuma. Se toma en mate o tereré)
71. Yerba mata (Para el corazón. Se toma con agua fría)

表－2

LISTA DE PLANTAS CULTIVADAS POR ESQUEJES

Nombre vulgar	No de plantascultivadas	Crecimiento (No)	Por ciento
1- Cambara	30	9	30.0%
2- Cangorosa	10	9	90.0%
3- Poleo	30	6	20.0%
4- Cocú	9	4	44.4%
5- Chirca melosa	30	12	40.0%
6- Ñangapyry	30	13	43.3%
7- Ambay	30	3	10.0%

FECHA DE PLANTACION: 4-5 de junio de 1985

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Ⅵ 植物化学での指導報告

富山医科薬科大　薬学部助教授
専門家　清　水　岑　夫　報告

派遣期間　1985. 5/10 － 7/9　2カ月

昭和60年5月10日より2ヶ月間植物化学部門の派遣専門家として、パラグアイ国に滞在中Asuncion市内第4市場で入手した薬草71種、薬草取扱商社（山脇商会）購入品16種及びAsuncion大学構内近くで採集した野生品4種の計91種について抽出したものから順次各種活性試験を行った（別表）。そのうちAldose Redctase阻害活性、抗ヒスタミン活性及びカラゲニン浮腫抑制効果（局所適用によるもののみ）に関する研究結果について報告する。

Ⅰ　Aldose Reductase(AR)阻害活性

糖尿病のような高血糖状態では細胞内のＡR活性のわずかな抗進でもaldoseからalditolへの還元が盛んとなり、その成績体の細胞内蓄積を助長する。その結果細胞異常を来し、白内障へと進行する。従ってこのＡR活性を阻害する物質に糖尿病の併発症の1つである白内障の治療あるいは予防効果を期待する事ができる。

本年度は29種のパラグアイ薬草エキスについてＡR阻害活性を調べた結果次の9種に強い活性が認められた。（表1）。

表1．ＡR阻害活性の認められたもの

Exp №.	植物材料	基源植物名	評価*（IC_{50} μg）
2	Sará moroti	Citharexylum myrianthum Cham.	++（2.2）
7	Týpýchá-kuratũ	Scoparia dulcis L.	+（4.9）
8	Tapecué	Acanthospermum australe O.K.	++（2.3）
12	Para-paraí mi	Phyllanthus niruri L.	++（1.1）
14	Marcela	Achyrocline satureioides(Lam.) DC.	+（3.1）
21	Eucalipto	Eucalyptus sp.	++（2.2）
26	Caá heé	Stevia rebaudiana Bert.	++（2.0）
28	Chirca melosa	Baccharis articulata Pers.	+（3.1）
29	Culantrillo	Adiantum cuneatum Langsd. et Fish.	+（4.5）

* 10 μg/mℓ の濃度で50％以上の阻害率を示し、IC_{50} ＜2.5 μg/mℓの場合 ++ とした。

抽出条件は70％ EtOH 温浸を原則とするが、アスンシオン大学の研究室に於ける初期の事情により一部は室温抽出したものもあり、上記材料中室温抽出エキスでも活性の認められたものはExp No: 2（＋、IC_{50} 5.0）、12（++、IC_{50} 1.0）、14（++、IC_{50} 2.4）、21（＋、IC_{50} 3.0）の4種である。

活性の認められたもののうちTapecueについて活性成分の単離精製を行った結果、天然では比較的分布の少ない5, 7, 4′－trihydroxy 3, 6－dimethoxy flavoneを得、強い阻害活性（IC_{50} 1×10^{-7}M）を認めた。これまでＡＲ阻害活性が強いと言われているquercitrinより強く、近年さらに強い活性を有するフラボノイドとして報告されたaxillarin及びLARI 1と比較しても、それらと同等もしくはそれ以上の活性が認められ、その詳細については日本薬学会（昭和61年4月、第106年会、千葉）で発表し投稿済である（論文コピー添付）。Tapecueはパラグアイでは血液の停滞、リュウマチ、関節炎、出血等の治療に利用されているが糖尿病やその併発症である白内障に効果があるという情報はない。

今回計らずも本植物にＡＲ阻害効果が認められた事より、糖尿病の併発症である白内障にも利用出来る可能性を見出した。今日数多くの利用されている医薬品の殆んどが天然から見出されたものであり、しかも経験的な薬効とは異なった活性が判明し、意外な薬物が生れた例は沢山ある事を考えた場合、今回TapecueからＡＲ阻害活性成分を見出した意義は大きく、今後さらにパラグアイで昔からグアラニーインディオによって伝承されてきた薬草を広く研究していく上で大きな期待が持たれる。

Ⅱ　抗炎症活性

1．局所適用によるカラゲニン浮腫抑制作用

薬草の中で腫れもの等にその生汁あるいは抽出エキスを外用して効果をあげている場合が多い。そこで、ラットに材料のエキスを塗布し、カラゲニンで誘発した浮腫に対する消炎効果を調べた。29種（Exp　№1～30）のうち次の5種に効果が認められた。（表2）

表2　消炎効果の認められたもの

Exp №.	植物材料	基源植物名	評価*（抑制率）
7	Typycha-kuratu	（表1参照）	++（46.8％）
8	Tapecue′	（　〃　）	++（61.7％）
12	Para-parai mi	（　〃　）	+（18.7％）
17	Burrito	Minthostachys mollis Kunth	++（31.1％）
19	Alhucema	Lavandula latifolia Vill.	+（18.9％）

＊　20 mg／ratで　＊$p<0.05$→＋、　＊＊$p<0.01$→＋＋

活性の認められたもののうち、Tapecueは、外用として腫れものに利用されている事から本エキスについて活性成分の解明を行った。その結果ｎ－ヘキサン可溶部に活性が集中し，カラムクロマト等による分離精製を行ったが量的に不足したため脂肪酸と ursolic acid を単離したにとどまった。

2．抗ヒスタミン作用

急性炎症の初期の段階に対する消炎効果をみる方法としてヒスタミンによるモルモット摘出回腸の収縮に対する抑制効果を調べた。29種のうち次の４種に効果が認められた。（表３）

表３　収縮抑制効果の認められたもの

Exp No.	植物材料	基源植物名	評価*
13	Mil hombre	Aristolochia trianglaris Cham. et Sch.	++
14	Marcela	Achyrocline satureioides (Lam.) DC.	++
16	Romero	Rosmarinus officinalis L.	++
28	Chirca melosa	Baccharis articulata Pers.	++

＊　１００μg／mℓで５０％以上の抑制率を示すものを＋、７０％以上の抑制率を示すものを＋＋とした。（今回＋は省略）

活性の認められたもののうち、Mil hombreは南米では毒蛇に噛まれたり、さそりにさされたりした時の予防としての効果がいい伝えられ、又外傷や腫れものにその煎液を塗布して用いるとよいとされている事からその有効成分の検討を行った。その結果活性クラクションより各種クロマトグラフィーにより化合物１－４を得た。これらは、いずれも本植物より単離報告されている galbacin, 4－hydroxy－3′－methoxy－3′, 4′－desmethylenedioxy galbacin, cubebin 及び 3′, 4′－dimethoxy－3′, 4′－desmethylenedioxy－cubebin と判明した。

これらの化合物について抑制効果を調べた結果、弱いながら２（IC_{50}　1.1×10^{-5}M）及び３（IC_{50}　1.3×10^{-5}M）に効果が認められた。（ちなみに抗スタミン剤の Dipenhydramine は IC_{50}　6.0×10^{-8}）

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Chemical and Pharmaceutical Studies on Medicinal Plants in Paraguay. I. Isolation and Identification of Lens Aldose Reductase Inhibitor from "Tapecué," *Acanthospermum australe* O.K.[1]

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The EtOH extract of "Tapecué," *Acanthospermum australe*, was found to have a potent inhibitory activity towards rat lens aldose reductase (AR). From the active fraction of the extract, 5,7,4'-trihydroxy-3,6-dimethoxyflavone was isolated. It was found to have higher activity ($IC_{50} = 1 \times 10^{-7}$ M) than quercitrin, which is a known inhibitor of AR ($IC_{50} = 1.8 \times 10^{-6}$ M in our bioassay).

Keywords——*Acanthospermum australe*; Compositae; 5,7,4'-trihydroxy-3,6-dimethoxyflavone; aldose reductase inhibitor; rat lens

There is a traditional system of medicine, "Medico de Yuyo," employing medicinal plants in Paraguay. In screening tests for biological activities of these plants "Tapecué," *Acanthospermum australe* (Compositae), showed weak inhibitory effects on β-glucuronidase activity and on the growth of KB cells and high inhibitory activity towards rat lens aldose reductase (AR). This paper deals with the isolation and identification of chemical constituents in "Tapecué," and identification of the active component inhibiting rat lens AR, which plays a significant role in the reduction of aldose to alditol under abnormal conditions such as diabetes.

"Tapecué" is an important crude drug which has traditionally been used for the treatment of blood stagnation, rheumatism and arthritis by internal administration, and of swelling and bleeding by external application in "Medico de Yuyo." Various diterpenes,[2] acanthospermal A, tridecapenta-3,5,7,9,11-yne-1-ene, thymol, isothymol, *etc.* have been isolated from this plant[3] but no studies in relation to the biological activity have been reported. Chemical and pharmacological studies of another plant of the same genus, *Acanthospermum glabratum*[4] have revealed no AR inhibitory activity.

EtOH : H_2O (7 : 3) extract (A) was suspended in water and extracted with *n*-hexane, $CHCl_3$ and *n*-BuOH successively to afford *n*-hexane extract (B), $CHCl_3$ extract (C), *n*-BuOH extract (E) and residue (F) (Fig. 1.)

The extract E (Table I), which was most active, was applied to a column of polyamide, and elution with MeOH : H_2O (3 : 2) followed by MeOH and $CHCl_3$ gave four fractions (fr. 1—4) (Fig. 1).

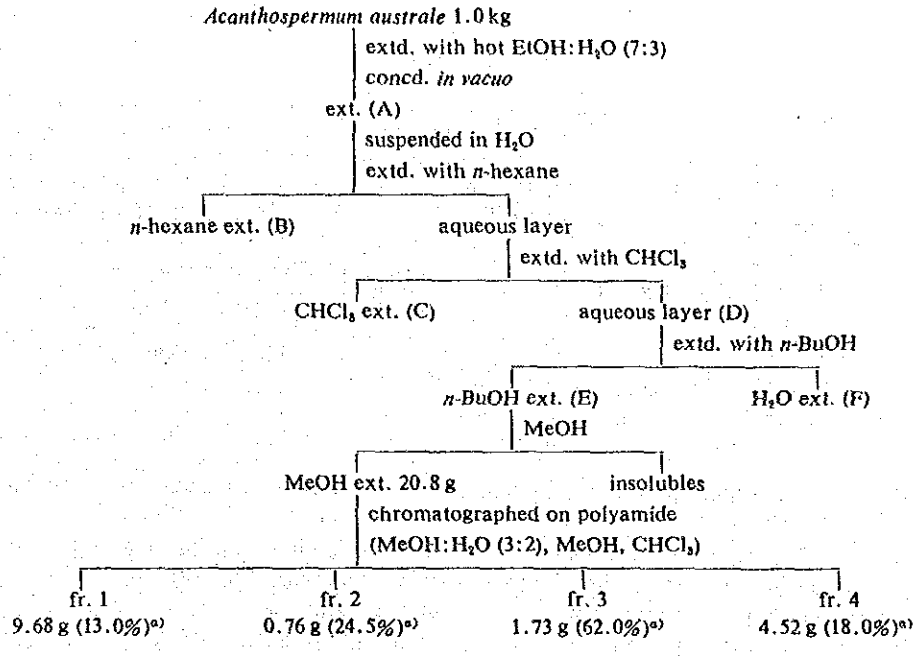

Fig. 1. Fractionation of Biologically Active Constituents of *Acanthospermum australe*

a) Values in parentheses indicate the inhibitory activities towards crude rat lens aldose reductase at the concentration of 1 μg/ml.

TABLE I. Inhibition of Crude Rat Lens Aldose Reductase by Extracts from *Acanthospermum australe* and Compounds **1**—**6**

Extract	IC_{50} (μg)	Yield (%)	Compound	IC_{50} (μM)
A	2.3	100	1	0.1
B	20.0	13	2	—
C	4.0	14	3	3.2
D	2.6	—	4	9.2
E	1.5	29	5	4.8
F	13.0	43	6	—
			Quercitrin[a]	1.8

a) Quercitrin was assayed previously, and was tested again as a reference in this study.

Three crystalline compounds **1**, **2** and **3** were obtained from fr. 3, which exhibited higher activity than other fractions, by gel-filtration and silica gel column chromatography. Compounds **4**, **5** and **6** were obtained from fr.2 and fr. 4.

Compound **1**, yellow needles, exhibited a positive reduction test for flavonoids. Infrared (IR) and ultraviolet (UV) spectra of **1** showed the characteristic absorption patterns of flavonoids. In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1**, peaks due to four aromatic protons appeared as A_2B_2 type signals attributable to B ring protons. Another aromatic proton signal at 6.6 ppm assigned to the C-8 proton and a 6H singlet at 3.8 ppm attributed to two methoxyl groups were observed. The presence of three hydroxyl groups at C-5, C-7 and C-4′ in **1** was determined by analysis of the UV spectrum.[5] From the above results, **1** was concluded to be 5,7,4′-trihydroxy-3,6-dimethoxyflavone[6] and this identification

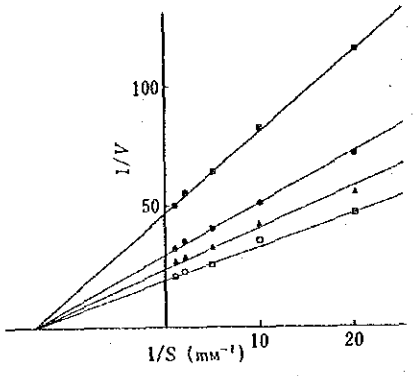

Fig. 2. Lineweaver-Burk Plots of Lens AR Activity

Enzyme activity was measured at each substrate concentration in the presence and absence of inhibitors. Key: (□) control, (●) in the presence of 10^{-7} M 1, (▲) 5×10^{-8} M 1 and (■) 10^{-6} M quercitrin. The substrate is glyceraldehyde (S) and the velocity units (V) are changes in OD_{340}/200 s.

was confirmed by comparison of the physical and spectral data with those of an authentic sample.

Compounds **2**, **3**, **4** and **5** were identified as trifolin, hyperin, rutin and quercetin, respectively, by comparison of the physical and spectral data with those of authentic samples.

Compound **6**, a pale yellow powder, exhibited a negative reduction test for flavonoids and a positive color reaction to $FeCl_3$ and was concluded to be caffeic acid from the physical and spectral data.

Inhibitory Effect on Crude Rat Lens AR

Compound **1**, which has not previously been tested for inhibitory activity towards AR, exhibited the highest activity ($IC_{50}=1.0\times10^{-7}$ M) among compounds **1**—**6** and was about 18 times more potent than quercitrin ($IC_{50}=1.8\times10^{-6}$ M) (Table I).

According to Okuda *et al.*,[7] axillarin and LARI 1 are the most potent inhibitors of aldose reductase known so far ($IC_{50}=5.2\times10^{-8}$ and 4.2×10^{-8} M), respectively, being at least 6 times more potent than quercitrin (3.1×10^{-7} M). Some flavonoids showed varying activities depending on the solvent used,[8] and different values of IC_{50} of quercitrin were found by Varma *et al.*[9] and Okuda *et al.*,[7] and in this work, so the comparative potency of compounds should be estimated under the same conditions. As judged from the relative potencies (IC_{50}) of compound **1**, axillarin and quercitrin, **1** might be as potent as or more potent than axillarin.

We concluded that compound **1** is mainly responsible for the rat lens AR inhibitory activity of this plant.

Kinetics of Inhibition by Compound 1

Kinetic studies were conducted with **1** in order to determine the type of inhibition and the inhibition constant (K_i). The Lineweaver-Burk plots are shown in Fig. 2. Compound **1** was found to be a non-competitive inhibitor at the concentrations of 1.0×10^{-7} and 5.0×10^{-8} M, as was seen in the cases of quercitrin[7] and axillarin,[7] but it did not show the same type of inhibition at the concentration of 5.0×10^{-7} M. Okuda *et al.*[7] reported that many uncompetitive inhibitors display non-competitive inhibition at low concentrations and switch to uncompetitive inhibition at higher concentrations. In our experiment, **1** showed a similar action. The K_i value of **1** for lens AR was 2.05×10^{-7} M.

The inhibitory effect of **1** on lens AR was also checked in the presence of a large amount of bovine serum albumin (BSA). Compound **1** showed almost the same degree of inhibition in the presence and absence of BSA, suggesting that **1** inhibits the activity of lens AR even in the presence of other proteins.

Experimental

The melting point is uncorrected. IR and UV spectra were obtained with Hitachi 260-10 and Hitachi 220S spectrometers. ^{1}H-NMR spectra were taken with a Hitachi R-24B (60 MHz) spectrometer with tetramethylsilane as an internal standard, and chemical shifts are given in δ (ppm). Mass spectra (MS) were obtained on a JEOL-JMS-D 200 instrument. Paper partition chromatography (PPC) was performed on Toyo filter paper No 51B employing the descending technique with AcOH : H_2O (15 : 85) and *tert*-BuOH: AcOH : H_2O (3 : 1 : 1) (TBA) as developing solvents, and the spots were detected under a *UV* lamp. Thin layer chromatography (*TLC*) was performed on Kieselgel $60F_{254}$ plates (Merck); spots were detected under a UV lamp and by heating after spraying 10% H_2SO_4.

Plant Materials——"Tapecué" was purchased from local dealers in Asunción., Paraguay and identified as *Acanthospermum australe* O.K. (aerial part) by Dr. H. Koyama, Faculty of Science, Kyoto University.

Bioassay——Crude AR was obtained from the supernatant fraction of the homogenate of rat lens according to the method of Kador and Sharpless.[10] One unit was defined as the amount catalyzing the oxidation of 1 μmol of reduced nicotinamide adenine dinucleotide phosphate per minute. Samples (1.4—2.0 units) were stored frozen until needed. The inhibitory effects of extract A—F and the isolated compounds on AR were assayed by the method previously reported.[8] Samples were dissolved in dimethylsulfoxide, which was found to have no effect on the enzyme activity at below 0.1% concentration.

Extraction and Fractionation——Dried powder (1 kg) of "Tapecué" was extracted with hot EtOH : H_2O (7 : 3) (1 h × 3). The EtOH : H_2O (7 : 3) solution was concentrated *in vacuo* to give the extract A (118 g). Extract A (100 g) was suspended in H_2O (600 ml) and extracted with *n*-hexane (500 ml × 3), $CHCl_3$ (800 ml × 3) and *n*-BuOH (670 ml × 3) successively to yield the biologically active extract E (29 g). The MeOH solubles (20.8 g) of E (21 g) was chromatographed on polyamide (Waco C-200, 280 g, 5 × 50 cm). Elution with MeOH : H_2O (3 : 2), MeOH and $CHCl_3$ gave fr. 1 (9.68 g), fr. 2 (0.76 g), fr. 3 (1.73 g) and fr. 4 (4.52 g). The most biologically active fr. 3 was subjected to gel-filtration (Toyopearl HW-40F) and silica gel column chromatography to give compounds 1 (13 mg), 2 (2 mg) and 3 (41 mg). From fr. 2, compounds 4 (21 mg) and 5 (7 mg) were obtained by column chromatography (silica gel and Sephadex LH 20). Compound 6 (110 mg) was obtained from fr. 4.

Compound 1 (5,7,4'-Trihydroxy-3,6-dimethoxyflavone)——Yellow needles, mp 199—200 °C ($CHCl_3$/MeOH). *PPC Rf* 0.34 (15% AcOH), 0.86 (*TBA*). *Mg+HCl*: orange; *Zn+HCl*; red-violet. *MS m/z*: 330 (M^+), 315. *UV* λ_{max}^{EtOH} nm (log ε): 341 (4.22), 270 (4.14). IR ν_{max}^{KBr} cm^{-1}: 3450, 1660, 1610.

Compounds 2—6——2, mp 236 °C (MeOH), 3, mp 233—234 °C (EtOH), 4, mp 192—195 °C (MeOH/H_2O), 5 mp > 300 °C, and 6, mp 220—222 °C (MeOH/H_2O), were identical with authentic trifolin, hyperin rutin, quercetin and caffeic acid, respectively.

Acknowledgement This work is a part of a joint study between Japan and the Republic of Paraguay on medicinal plants in Paraguay supported by both governments through the Japan International Cooperation Agency (JICA). We wish to thank Prof. T. J. Mabry, University of Texas at Austin, Texas, U.S.A., for providing 5,7,4'-trihydroxy-3,6-dimethoxyflavone. We also wish to thank Dr. H. Koyama, Faculty of Science, Kyoto University, for identification of *Acanthospermum australe* O.K.

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1986. 1. 29
別　表

10μ/mℓ 50% 以上　100μ/mℓ 50% 以上　IC_{50}<5μ/mℓ No.31～ 10μ/mℓ→70%以上+、90%以上++)　IC_{50} <10μ/mℓ　IC_{50}<50μ/mℓ　ED_{50}<50μ/mℓ　ED_{50}<50μ/mℓ (<100μ/mℓ±)　20 mg/rat ＊→+ ＊＊→++　100μ/mℓ >50%+ >70%++

Material Extracted ＼ 上段 倒表(h) 下段 室温(r) Assay			Aldose Reducase (IC_{50})	Angiotensin-converting enzyme	β-Glucuro-nidase	Xanthine Oxidase	Urease	KB Cell	L5178Y Cell	Inflammation	Histamine	Diabetes
1. AMBAŶ	Ceropia adenopus Mart	hot (h) room (r)										
2. SARÁ MOROTÍ	Citharexylum myrianthum Cham	h	++(2.2×10^{-5})						(N.T)			
		r	+(5.0×10^{-6})					+(54μ)	+(37μ)			
3. PIPER Sp. №2 (Hojas lunceoladas)									+			
4. PIPER Sp. №1 (Hojas grandes anchas)		r								+?		
5. ÑANGAPIRŶ	Eugenia uniflora L.	h				+(6.6μ)						
6. CEDRÓN-CAPIÍ	Andropogon aitratus	h		+(50%)				±	+(34μ)			
		r										
7. TŶPŶCHÁ-KURATŨ	Scoparia dulcis L	h	+(IC_{50} 4.7×10^{-6})		+(5.4)			±		++		
		r						+(52μ)	+			
8. TAPECUÉ	Acanthospermum australe O.K.	h	++(IC_{50} 2.3×10^{-6})		+(4.0)			+(46μ)		++		
9. KOKÚ	Allophylus edulis Radrk.	h			+(5.0)			±				++
10. COLITA	Cordia salicifolia Cham.	h		+(50%)		±(9.5μ)						
		r			+(5.0)			±	±(65μ)			
11. ŶVA HAI	Eugenia myrcianthes Niedenzu	h			+(5.0)	+(2.5μ)	+(28μ)					
12. PARAPARAI MÍ	Phyllanthus niruri L.	h	++(IC_{50} 1.1×10^{-6})						(N.T)	(+) 18.7%(3h)	( )	
		r	++(IC_{50} 1×10^{-5})	+(32%)		±		±(44μ)	+(21μ)	+		
13. MIL HOMBRE	Aristlochia triangularis Chem. et schleht	h		( )	( )	( )	( )	( )	(N.T)		++	
		r							+(18μ)		++	
14. MARCELA	Achyrochine satureioides (Lam) DC.	h	+(3.1×10^{-5})		++(10μ-86.3%)	++(50μ-94%)		+(50μ-66.8%)	(N.T)		++	
		r	++(2.4×10^{-5})			±(17μ)		±(72μ)	+(34μ)		+	
15. COLA DE CABALLO	Equisetum giganteum L.	h				±(50μ-59.8%)			(N.T)		( )	
		r						+(53μ)				
16. ROMERO	Rosmariuns officinalis L.	h			±(filt)	+(filt)	+		(N.T)		++	
		r					++(10.5μ)	+(50μ)	+(50μ)			
17. BURRITO	Minthostachys mollis Kunth								(N.T)	(++) 31.1%(1h)	( )	
18. YAGUARUNDI	Piper fulvencens C.D.C. =PIPER Sp. №1	h			±				(N.T)	h	( )	
		r							±(55μ)	r		
19. ALHUCEMA	Lavandula latifolia Vill.	h			±				(N.T)	23.2 +18.9%(4h)	( )	
20. ESPARTILLO GUAZU	Elinorus latiflorus	h							(N.T)	N.T	( )	
21. EUCALIPTO	Eucalyputus globulus	h	++(2.2×10^{-5})		+			+(50μ 78.2%)	(N.T)	N.T	( )	
		r	+(3.0×10^{-6})					+(50μ 78.2%)				

Material Extracted \ Assay		Aldose Reductase	Angiotensin-converting enzyme	β-Glucuronidase	Xanthine Oxidase	Urease	KB Cell	L5178Y Cell	Inflammation	Histamine	Diabetes
22. CANGOROSA	Maytenus ilicifolia Mart					±					
23. SIETE, SANGRIA(S)	Cuphea racemosa (L.f.) Spreng										
24. CEDRON PARAGUAY	Lippia triphylla Kuntze										
25. SIEMPRE VIVA	Gomphrena parennis L.			+(10μ—70%)							
26. KAA HEE	Stevia rebaudiana Bert.	++(2.0×10^{-5})		±							
27. AROMITA	Acacia farnesiana (L) Willd				+(50μ—77.3%)		-				
28. CHIRCA MELOSA	Baccharis articulata Pers	+(3.1×10^{-5})		+(10μ—76.9%)						++	
29. CULANTRILLO	Adiantum cuneatum L.	+(4.5×10^{-5})							N.T		
30. PIPER Sp. №3											
31. CAMBARA	Moguinia polymorpha Cab.	+(3.0×10^{-5})		+				N.T	N.T		
32. PARATODO (PIRE)	Tabebuia caraiba Mart.								N.T		
33. ARACHICHU	Solanum nigrum L.								N.T		
34. ALTAMISA-(ITE)	Ambrosia artemisifolia L.						+(50μ—81%)		N.T		
35. CABELLO DE ANGEL	Cusenta xanthochortos Fugelm								N.T		
36. CAATAI	Polygonum acre H.B.K.								N.T		
37. CAARE { aerea (RAIZ)	Chenopodium ambrosioides L.						?(50μ—69%)		N.T		
38. SAUCO	Sambucus australis Chem. et Sch.								N.T		
39. MOLLE-I		+							N.T		
40. SALVIA		+							N.T		
41. CARAGUATÁ RUÂ	Eryngium floribundum Cham								N.T		
42. PINDÓ (RAPO)	Arecastrum romamzoffianum Bece.						±(50μ—57%)		N.T		
43. ÎSÎ	Protium heptaphyllum (Anth) Mart.	±							N.T		
44. GUAYACAN (CORTEZA)	Caesalpinia melanocarpa Griseb.	+	+(41%)						N.T		

Material Extracted \ Assay		Aldose Reductase	Angiotensin-converting enzyme	β-Glucuro-nidase	Xanthine Oxidase	Urease	KB Cell	L5178Y Cell	Inflammation	Histamine	Diabetes
45. PERDUDILLA NEGRA											
46. MALVA BLANCA	Sida cordifolia L.						?(50μ-67%)		N.T		
47. GUAYABA	Psidium guajava L.	+							N.T		
48. TAPERŶVÁ-HÚ	Cassia occidentalis L.		+(34%)						N.T		
49. PENICILINA											
50. VERBENA-Í	Verbena bonariensis L.	±		+					N.T		
51. TAMANDÁ CUÑÁ	Catasetum barbatum Lindle								+(1hr 30.2%, 2hr 20.0%)	++(50%) (diphen. 7.2×10^{-8})	
52. AGUAPÉ PURUA	Eichhornia crassipes (Mart) Solms								N.T		
53. ŶVŶRÁ PŶTÁ(AEREA)	Peltophorum dubium (Spreng) Tamb.		+(52%)	+		+			N.T		
54. ALCANFOR DEL CAMPO		+							N.T		
55. CALAGUALA	Polypodium phyllitidis L.								N.T		
56. ŶVOPÉ	Gleditsia amorphoides Taub.						++(50μ-89%)		+		
		finished	finished	finished		finished	finished	not tested	N.T not tested	finished	not tested

Ⅶ　植物化学での指導報告

富山医科薬科大学薬学部

専門家　有　澤　宗　久　報告

派遣期間　　1985．5/10～7/9　2ヶ月

1．任国配属機関での研究室の整備、ならびに研究機材の搬入およびそれらの据付調整。

1ヶ月赴任の鈴木、2ヶ月赴任の清水両専門家と供に行った。これら搬入据付調整の資機材のリストは P．102～108を参照されたし。

2．任国配属機関における研究材料の調達および伝承薬物の予備調査

前記両専門家と供にアスンシオン市内のスーパーマーケット、薬局などの予備的情報を得たのち第4市場における薬草の予備調査、研究材料の調達、郊外への薬草採集、写真撮影などを行った。 P．109～111 を参照。

3．任国配属機関における薬草エキスの調整およびその指導

加熱抽出装置が未着であったので冷浸法によって抽出した。即ち、75％エタノールにて材料を温室で浸出し、浸出液を得、これをロータリー・エバポレーター装置にて減圧下、エタノールを溜去してエキスを得た。赴任中、清水専門家と供に2人の女子カウンターパートに指導しながらP.111記載の20種の薬草エキスを調整した。

4．カウンターパート（ルシア・フランコ）に対する本邦所属先での研究指導

（S 60．7．26～10．3）

(1)　化学成分の単離および構造解明

生薬槐花からルチンを単離させ構造解明のトレーニングを行ったが、この過程の中で下記に列記する技術指導を行った。

① 還流装置を用いた抽出法

② 濾過方法（必要に応じた濾過を行うこと、ヒダ濾紙の折り方など）

③ 減圧濃縮方法

④ 呈色反応（フラボン反応、塩化第2鉄反応など）

⑤ ペーパー・パーチション・クロマトグラフ（P．P．C）法の利用

⑥ 薄層クロマトグラフ（TLC）法の利用

⑦ 加水分解方法

⑧ アセチル化方法

⑨ 融点測定方法

⑩ 赤外線吸収（IR）スペクトル測定方法

⑪ UV スペクトルの構造解明への応用

⑫ IR スペクトルの構造解明への応用

⑬ 核磁気共鳴（NMR）スクペクトの構造解明への利用

(2) アンジオテンシン変換酵素（ACE）阻害試験

ACE の血圧調整機構の関与について教育指導を行ったのち本試験法を行い下記の技術指導を行った。

① pH メーターの使用方法

② 緩衝液の調整

③ ACE 原液の調整

④ 蛍光光度計の取扱い方

⑤ 蛍光光度の測定方法

⑥ ACE 阻害試験法の手順

⑦ 標準阻害剤（カプトプリル）を用いた ACE 阻害試験法

5．生物活性試験

(1) ACE 阻害試験

P.112～113 の試験方法に従って薬草エキスについて実施し、別表（P.97～99）の様な結果を得た。

(2) 細胞毒性試験

人の咽喉癌由来の KB 細胞とマウス白血病由来の L 5178 Y 細胞に対する増殖抑制について P.114～119 の試験方法に従って実施し、別表（P.97～99）の様な結果を得た。

JAPAN INTERNATIONAL COOPERATION AGENCY

P. O. Box 216, Mitsui Bldg., Shinjuku-ku, Tokyo, Japan.

Nos.	Description of	Quantity	Unit Price	Amount
1	COPY MACHINE (CANON NP-155)	1 set		676,000
	*CASSETTE (B4, B5, A4)			
	*COPY PAPER (B4/2box)(B5/2box)			
	(A4/2box)(A5/2box)			
	*STAND (WITH TONNER, 8pcs)			
2	CAMERA (ERUMO 2600AF)	1 set		126,000
	*MICROPHONE (EC-205)(1pc)			
	*LENS HOOD CASE (1pc)			
3	PROJECTOR (100V ERUMO SC-30)	1 set		154,000
4	FILM (KODACK KMA-594)	50 pcs	@¥ 1,700	85,000
5	FILM (KODACK ELA-594)	50 pcs	@¥ 2,000	100,000
6	FILM (KODACK KMA-580)	5 pcs	@¥ 4,450	22,250
7	SLIDE PRCJECTOR (220V AS3000A)	1 set		142,000
8	TYPEWRITER (OLIVETTI P-35)	1 set		115,000
	with CARBON RIBBON/2pcs			
	with TRANSE /1pc			
	with LIFT OFF TAPE/1pc			
9	PERSONAL COMPUTER (NEC PC-9801 F2)	1 set		358,000
10	COLOR DISPLAY (PC-KD 551K)	1 set		89,000
11	SYRIAL PRINTER (NM-9400S)	1 set		279,000
12	FLOPPY DISIC (PC-9836-4)	1 box		13,500
13	PRINTER PAPER (T-1513 1P)	1 box		6,000
14	RIBBON (NM-9004-001)	4 pcs	@¥ 2,000	8,000
15	AVR TRANSFORMER (1 kw)	1 pcs		150,000
16	ELECTRONIC DISPENSING BALANCE (PE-11)	1 pc		325,000
17	ELECTRONIC DISPENSING BALANCE (11712 MP-8)	1 pc		590,000
18	REFRIGERATOR (SR-521BF)	2 pcs	@¥150,000	300,000
19	AUTOMATIC WATER DISTILLATION	1 pc		784,800
	APPARATUS "AQUARIUS" GSR-27			
20	CENTRIFUGE (H-103NR)	1 pc		576,000
21	ROTARY EVAPORATOR (RE-51-A4)	2 pcs	@¥216,800	433,600
22	HANDY ASPIRATOR (JS-27K)	2 pcs	@¥ 66,800	133,600
23	WATER BATH (WH-12)	1 pc		66,000
24	HOTTING BATH (B-UP)	1 pc		20,000
25	LABORATORY JACK (30 × 30cm)	1 pc		29,600
26	MAGNETIC STIRRER (D-2S)	1 pc		43,000
27	MANTLE HEATER (AFS-50)	1 pc		54,000

Nos.	Description of Goods	Quantity	Unit Price	Amount
28	PH METER (F8DP)	1 pc		330,000
29	BATH, CONSTANT TEMPERATURE (ET-80)	1 pc		420,000
30	MILLS, WIREY (1029-B)	1 pc		220,000
31	GAS BURNER LPG	1 pc		19,500
32	TEST TUBE MIXER (TME-21)	1 pc		25,000
33	UV DETECTOR (CL-15)	1 pc		69,000
34	UV DETECTOR (UV -15)	1 pc		115,000
35	FORCED CONVECTION OVEN (FC-42T)	1 pc		356,000
36	Measuring Cylinder 200㎖	2 pc	@¥ 1,160	2,320
37	-do- 100㎖	2 pc	960	1,920
38	Measuring Pipette 10㎖	10 pc	340	3,400
39	-do- 5㎖	10 pc	270	2,700
40	-do- 1㎖	10 pc	200	2,000
41	Triangle Flask 1,000㎖	5 pc	880	4,400
42	-do- 300㎖	10 pc	350	3,500
43	-do- 50㎖	10 pc	270	2,700
44	Beeker 300㎖	10 pc	260	2,600
45	-do- 100㎖	10 pc	200	2,000
46	Washing Machine for Pipette	1 pc		13,000
47	Glass Flask 60 φ	3 pc	350	1,050
48	-do- 105 φ	3 pc	660	1,980
49	-do- 180 φ	3 pc	1,500	4,500
50	Filter Paper №2, 125/100 sheets	3 box	440	1,320
51	"SUNPU" Set M-type	1 pc		2,000
52	"SUNPU" № 1 Liquid 50㎖	1 pc		600
53	"SUNPU" B-board/30 sheets	10 pc	250	2,500
54	"SUNPU" Sheet/100 sheets	3 pc	680	2,040
55	Glass Board for Electrophoresis Spencer 2m/m	3 pc	12,000	36,000
56	Spencer 1m/m	1 pc		12,000
57	Coam 2m/m, 13-kentai	2 pc	5,000	10,000
58	Centrifuge Tube 15×105N	100 pc	29	2,900
59	Test Tube Stand 16.5×50 pcs	4 pc	1,500	6,000
60	Silicone Tube 25㎜φ	5 m	160	800
61	-do- 2.0㎜	5 m	120	600
62	Milk Syringe (Ceramic)	20 pcs	300	6,000
63	Measuring Cylinder 1000㎖	2 pcs	4,800	9,600
64	-do- 500㎖	2 pcs	2,240	4,480
65	Filter Paper № 2 22.5 φ	3 pcs	1,350	4,050
66	-do- 360 φ	3 pcs	2,700	8,100
67	Spartel 150m/m	2 pcs	130	260

Nos.	Description of Goods	Quantity	Unit Price	Amount
68	-do- 240m/m	2 pcs	290	580
69	Micro. Spartel	2 〃	160	320
70	Stenless Forceps 125	2 〃	130	260
71	Silicon Teat 5㎖	10 〃	180	1,800
72	Vvnil Bag 0.03×120×170/100 sheet	3 〃	900	2,700
73	"KIMU WAIPU" S-200	1 〃		11,000
74	Aluminume Foil 30㎝×5m	3 〃	3,000	9,000
75	Glass Tube	10 〃	240	2,400
76	Rubber Tube 12㎜×17㎜	5 〃	3,800	19,000
77	Wrapping paper for Medicien/500 sheets	5 〃	590	2,950
78	Gauze 30㎝×10m	5 〃	680	3,400
79	Glass Stirring Rod	10 〃	130	1,300
80	Plastic Bukets 15L	5 〃	1,100	5,500
81	Cleaning Plastic Bottle 500㎖	3 〃	170	510
82	Loupe 20X	5 〃	4,500	22,500
83	KJELDAHI Type Flaks 100㎖	5 pe	2,600	13,000
84	-do- 200㎖	5 〃	2,650	13,250
85	-do- 300㎖	5 〃	2,850	14,250
86	-do- 500㎖	5 〃	3,300	16,500
87	-do- 1L	3 〃	3,650	10,950
88	-do- 2L	3 〃	5,250	15,750
89	-do- 3L	3 〃	6,650	19,950
90	Measuring Cylinder 100㎖	2 〃	1,350	2,700
91	-do- 200㎖	1 〃		1,680
92	-do- 500㎖	1 〃		3,040
93	-do- 1L	1 〃		6,400
94	Flask 5㎖	3 〃	1,360	4,080
95	-do- 10㎖	3 〃	1,360	4,080
96	Measuring Pipette Tip 0.5㎖	2 〃	510	1,020
97	-do- 1㎖	2 〃	295	590
98	-do- 2㎖	2 〃	295	590
99	-do- 5㎖	2 〃	375	750
100	-do- 10㎖	2 〃	485	970
101	Silicon Pipette ㎜ 3	10 〃	100	1,000
102	-do- ㎜ 5	10 〃	150	1,500
103	Silicon Pipetter ㎜ 10	1 〃		3,000
104	-do- ㎜ 25	1 〃		3,000
105	Plastic Bottle Washer 500㎖	3 〃	170	510
106	TRAP Ball 29/42	1 〃		8,125
107	-do- 29/42 × 15/25	1 〃		7,850

Nos.	Description of Goods	Quantity	Unit Price	Amount
108	Liquid Dividing Funnel Cone 500㎖	2 pc	5,650	11,300
109	−do− 1 L	2 〃	9,650	19,300
110	−do− 2 L	2 〃	12,500	25,000
111	−do− 3 L	2 〃	18,300	36,600
112	KOMAGOME Pipette 2㎖	5 〃	100	500
113	−do− 3㎖	3 〃	150	450
114	Glass Cutter	1 〃		4,200
115	Red Liquid Thermometer 0−100℃	3 〃	250	750
116	Holder for Tefron Meter	2 〃	2,600	5,200
117	Loupe 20X	1 〃		4,500
118	Ring 120	2 〃	1,150	2,300
119	−do− 85	2 〃	600	1,200
120	Funnell 100 × 9 × 100	3 〃	830	2,490
121	−do− 75 × 8 × 75	3 〃	500	1,500
122	−do− 50 × 8 × 65	3 〃	420	1,260
123	−do− 180φ	3 〃	1,580	4,740
124	−do− 300φ	2 〃	10,400	20,800
125	Glass Stick 8m/m × 1,200m/m	5 〃	180	900
126	Glass Tube 8φ × 1,200m/m	15 〃	100	1,500
127	−do− 10φ × 1,200m/n	5 〃	135	675
128	ELECTRONIC DISPENSING BALANCE PE−11TYPE	1 set		280,000
129	REFRIGERATOR WITH TRANSFORMER SR5218F(A)	1 〃		140,000
130	CENTRIFUGE H−103NR	1 〃		547,000
	*HOLDER, 15㎖ 3,500R.P.M. (32pcs)			
	*−ditto− 50㎖ 4,000R.P.M. (4pcs)			
	*1.5㎖ SETTLING TUBE (50pcs)			
	*50㎖ SETTLING TUBE (8pcs)			
	*BALANCER (1pc)			
131	MAGNETIC STARER	1 〃		61,000
132	TEST TUBE MIXER TM−100 WITH TRANS	1 〃		24,000
133	SLAB GEL ELECTROPHOREST APPARATUS	1 〃		86,000
	SPG−1500W			
134	POWER SUPPLIES FOR ELECTROPHORESIS	1 〃		123,000
	*ELEPOS PS−1510			
135	MICRO CYLINGE	1 pc		6,300
136	PH METER F−80P	1 set		313,000
137	HYDROGEN PEROXIDE (500g)	1 pc		300
138	ACRYLAMIDE MONOMER (500g)	2 pcs		7,300
139	N, N′−METHYLENEBISACRYLAMIDE (SP25g)	2 〃		5,500
140	N, N, N′, N′ TETRAMETHYLETHYLENDIAMINE(100g)	1 pc		4,300

Nos.	Description of Goods	Quantity	Unit Price	Amoumt
141	VITAMIN B2 (1g)	1 pc		700
142	2-MERCAPTOETHANOL (25g)	1 〃		2,000
143	BROMOPHENOL BLUE (25g)	1 〃		3,000
144	POLYVINYL PYRROLIDONE K-30 (25g)	1 〃		550
145	COOMASSIE BRILLIANT BLUE R-250 (25g)	1 〃		4,000
146	UREA (500g)	1 〃		330
147	CHACOAL ACTIVATED POWDER (500g)	1 〃		1,330
148	RIVERSAL COLOR FILM	50 pcs	@¥ 2,000	100,000
149	ACETONE (500mℓ)	6 〃	@¥ 550	3,300
150	METHYL ALCOHOL (500mℓ)	18 〃	@¥ 400	7,200
151	HYDROCHLORIC ACID (500mℓ)	5 〃	@¥ 470	2,330
152	ACETIC ACID (500mℓ)	5 〃	@¥ 800	14,000
153	SCAT-20x-N (2KGS)	1 pc		3,300
154	SODIUM DODECYLSAL FATE (500g)	1 〃		7,000
155	DOTITE TMBZ (5g)	1 〃		22,000
156	TRIS(HYDROXYMRTHYL)AMINOMETHANE(500g)	2 pcs	@¥ 5,300	10,500
157	GLYCINE (AMINOACETIC ACID) (500g)	5 〃	@¥ 2,300	11,500
158	GLYCERIN (500mℓ)	2 〃	@¥ 1,250	2,500
159	SODIUM ACETATE CRYST (500g)	1 pc		330
160	AMMONIUM PERSULFATE (100g)	1 〃		300
161	STAINLESS MICRO SPARTEL 210	5 pcs	@¥ 160	300
162	-ditto- 180	5 〃	@¥ 160	300
163	STAINLESS SPOON' 165	3 〃	@¥ 60	180
164	-ditto- 210	3 〃	@¥ 175	325
165	STAINLESS TWEEZERS 150	3 〃	@¥ 150	430
166	-ditto- 130	3 〃	@¥ 180	340
167	VACUUM GUM TUBE 6 × 18	10 m	@¥ 1,060	10,300
168	FLASK 300mℓ	10 pcs	@¥ 400	4,000
169	-ditto- 500mℓ	10 〃	@¥ 580	5,300
170	-ditto- 1L	5 〃	@¥ 1,080	5,400
171	-ditto- 2L	3 〃	@¥ 2,200	6,500
172	-ditto- 3L	2 〃	@¥ 2,900	5,300
173	STIRRING PICKUP ROD TEFLON	1 pc		2,650
174	REAGENT BOTTLE 250mℓ	5 pcs	@¥ 1,500	7,500
175	-DITTO- 500mℓ	5 〃	1,900	9,500
176	-DITTO- 1L	3 〃	3,400	10,200
177	TEFLON STIRRING BARS 5 × 15	1 pc		330
178	-DITTO- 7 × 20	1 〃		320
179	-DITTO- 8 × 30	1 〃		350
180	FLASIC STAND 105φ	3 pcs	1,150	3,450

Nos.	Description of Goods	Quantity	Unit Price	Amount
181	-DITTO- 120φ	3 pcs	@¥ 1,150	3,450
182	JOINT CLAMP 15	5 〃	270	1,350
183	-DITTO- 29	3 〃	680	2,040
184	DIVIDE TUBE	2 〃	9,500	19,000
185	DIVIDE ADAPTER	2 〃	9,100	18,200
186	INDUCE ADAPTER	2 〃	9,100	18,200
187	DIVID TUBE	1 pc		25,000
188	CONDENSERS	2 pcs	15,000	30,000
189	BALL JOINL A	2 〃	3,900	7,800
190	-DITTO- B	2 〃	3,900	7,800
191	JOINT, SEPARATING	2 〃	3,900	7,800
192	OUALITATIVE FILTER PAPER 150φ	3 boxes	540	1,620
193	-DITTO- 300φ	3 〃	1,600	4,800
194	FILTER PAPER	1 box		5,400
195	PH TEST PAPER	1 〃		740
196	DEVELOPMENT TANK PAPER CHROMATOGRAPH	4 pcs	19,000	76,000
197	DYEING BAT	3 〃	1,000	3,000
198	TURN COLOR REACTION BOARD 2 × 6	2 〃	700	1,400
199	THREE-LEGGED STAND (M)	5 〃	2,500	12,500
200	-DITTO- (L)	3 〃	3,350	10,050
201	-DITTO- (LL)	3 〃	5,400	16,200
202	STAINLESS CAGE FOR TEST TUBE (200 × 200 × 200)	3 〃	3,300	9,900
203	STAINLESS CAGE FOR TEST TUBE (300 × 250 × 300)	2 〃	9,900	19,800
204	GLASS SPRAYER 30㎖	2 〃	2,600	5,200
205	POLYETHYLENE BOTTLE 2 L	5 〃	280	1,400
206	-DITTO- 3 L	5 〃	420	2,100
207	-DITTO- 5 L	5 〃	600	3,000
208	-DITTO- 10 L	5 〃	1,120	5,600
209	MANTLE HEATER 3 L	2 〃	27,000	54,000
210	POLYETHYLENE SIPHON	5 〃	250	1,250
211	PLASTIC BUCKET 10 L	3 〃	850	2,550
212	-DITTO- 15 L	1 pc		1,100
213	PLASTIC TUB 11 L	3 pcs	950	2,850
214	BUSKET SHALLOW TYPE	2 〃	550	1,100
215	BUSKET DEEP TYPE	2 〃	700	1,400
216	SCAR ANGLE TYPE BOTTLE 50㎖	10 〃	100	1,000
217	-DITTO- 100㎖	10 〃	110	1,100
218	-DITTO- 250㎖	10 〃	130	1,300

Nos.	Description of Goods	Quantity	Unit Price	Amount
219	RASP	1 pc		2,200
220	SECTIONAL STAND A TYPE	1 〃		33,500
221	SLYDUX	2 pcs	@¥ 36,000	72,000
222	PIPETTE MAN P-5,000	1 pc		45,000
223	-DITTO- P-1,000	1 〃		36,000
224	-DITTO- P- 200	1 〃		36,000
225	MICRO DISPENSER	1 〃		37,500
226	PIPETTE MAN CHIP C- 20	1 〃		11,250
227	-DITTO- C- 200	1 〃		11,250
228	-DITTO- C-6,000	1 〃		20,000
229	GAUZE 30㎝ × 10 m	3 pcs	@¥ 680	2,040
230	CAPILLARY TUBE FOR DISPENSER	1 pc		3,500
231	ALUMINIUM FOIL 30㎝ × 25㎝	3 pcs	1,800	5,400
232	COTTON 500g	3 〃	1,400	4,200
233	WIPE S-200	1 pc		11,000
234	STAINLESS WASHING CAGE	2 pcs	3,500	7,000
235	STAINLESS BLUSH ㎜ 4	5 〃	120	600
236	-DITTO- ㎜ 10	3 〃	140	420
237	MEDICINE WRAP PAPER	1 pc		500
238	CONE TYPE SETTLING TUBE WITH STOPPER	20 pcs	880	17,600
239	GUM TUBE 12 × 17 m/m	2 roll	3,400	6,800
240	TEST TUBE WITH STOPPER	50 pcs	510	25,500
241	STAINLESS TEST TUBE STAND 15 × 50	2 〃	1,500	3,000
242	-DITTO- 165 × 50	2 〃	1,500	3,000
243	PARA FILM	1 pc		3,200
244	TEST TUBE WITH STOPPER 20φ × 125	50 pcs	210	10,500
245	CHEMT TUBE 7 × 10	10 m	450	4,500
246	-DITTO- 8 × 11	10 〃	540	5,400
247	SODIUM CHLORIDE (500g)	1 pc	700	700
248	POTASSIUM CHLORIDE (500g)	1 〃	670	670
249	SODIUM BICARBONATE	1 〃	1,310	1,340
250	SODIUM PHOSPHATE, DIBASIC, CRYST	1 〃	700	700
251	POTASSIUM DIHYDROGEN PHOSPHATE	1 〃	970	970
252	HYDROCHLORIC ACID SOLUTION (500g)	1 〃	570	570
253	SUL PHURIC ACID (500g)	1 〃	440	1,440
254	ENZYM	1 unit	43,880	43,830
255	DIMETHYL SULFOXIDE (500g)	1 pc	1,590	1,580
256	SODIUM HYDROXIDE, SOLID (500g)	1 〃	540	540
257	POTASSIUM HYDROXIDE SOLID (500g)	1 〃	610	640
258	CALCIUM CHLORIDE (500g)	1 〃	990	990

21 de mayo de 1985.

PLANTAS MEDICINALES DEL PARAGUAY

1. Santa Lucía blanca (Refrescante) Conhelia nudiflora.
2. Cepa caballo (Refrescante. Se machaca y toma en el agua)
3. Perdudilla (Refrescante. En agua fría) Gonphrena decumbens.
4. Zarzaparrilla (Refrescante. En agua fría) Smilax orficinalis.
5. Cola de caballo (Para los rinones. En agua caliente) Xanthium spinosum. Equisetum giganteum.
6. Aguapé puruá (Para la inflamacion del estomago. En agua caliente)
7. Cocú (Refrescante. En agua fria) Allophyllus edulis.
8. Typycha curatú (Para indigestiones) Scoparia dulcis.
9. Nangapyry (Para adelgazar) Eugenia uniflora.
10. Capii cati (Refrescante)
11. Mbocaya-i rapo (diurético)
12. Eatatilla (Refrescante)
13. Raíz de perejil* (Abortivo)
14. Cedrón Paraguay (Para calmar los nervios) Lippiacitriodora
15. Llanten (Raíz) (Remedio para todo. Caliente)
16. Menta-í* (Para calmar los nervios)
17. Taropé (Refrescante - oll part) Dorstenia brasiliensis.
18. Tupasy camby (Refrescante)
19. Poleo-í (Remedio caliente en te o mate - abortivo)
20. Toronjil* (Para el corazón)
21. Nuaty pytá (Para
22. Urusu caty (Para echar lombrices - en decocción)
23. Verbena-í (Para dolor de garganta - en decocción)
24. Cerdon capii (Para calmar los nervios - té)
25. Raiz de hinojo (Para dolor de estómago. En agua caliente)
26. Toyuya (Abortivo. Se toma en tereré o mate)
27. Ysypo mil hombre (Abortivo y refrescante. En el mate) (Aumenta virilidad) Aristolochia trianguar.
28. Malva rapo pire (Abortivo. Decocción o té)
29. Cana brava (Abortivo. Decoccion o te)
30. Yagua Rova (Abortivo. Se toma té o decocción)
31. Ruibarbo (Abortivo. Se toma te o decoccion)
32. Charmáa caa (Para el estómago, para despertar el apetito)
33. Achicoria (Purgante. En té o decoccion)
34. Pindo rapo (Abortivo. En té o decoccion)
35. Para todo pire (Uso desconocido) Tecoma aregentes.
36. Usuru mee (Para catarro. En mate o té)
37. Guayacan corteza (Dolor de barriga. En té o decocción)

38. Ybyrá pytá piré (Para lavar heridas o problemas de la piel. Se cocina y lava)
39. Yateí caa (Remedio caliente. En té o decocción)
40. Yaguareté caa (Para el estomago. En té o decocción) (Como depurativo)
41. Calaguala (Abortivo. En té o decocción o en mate)
42. Penicilina (Para limpiar heridas. Se hierve y lavar)
43. Doradilla (Abortivo. Té o decoccion)
44. Flor de mamón macho (En forma de jarabe para la tos de los ninos especialmente)
45. Cangorosa (Abortivo y para úlcera. Té o decocción)
46. Malva blanca (Para eliminar catarro. Té o decocción)
47. Yuruveva Té o decocción)
48. Ambay (Para la tos, catarro. Té o decocción)
49. Yerba de lucero (Para el estómago. Té o decocción)
50. Pata de buey (Para los riñones. En tereré o mate)
51. Yaguá rundy (Para la tos. Te o decocción)
52. Sauco (Para inflamación del estómago. Friccionar)
53. Alcanfor del campo En mate)
54. Yateí caá (Remedio refrescante. En tereré)
55. Barba de maíz (Avatí zogüé) (Para los rinones. En té o mate)
56. Savá morotí corteza (Para diabetes. Se machaca, sa hierve y se toma como agua)
57. Macho acá raíz (Para el corazon. Se machaca y se toma con agua fría)
58. Para para-i (Para los rinones, rompe piedras. Con agua fria o caliente)
59. Siete sangría (Para la presión. Con agua fría o en mate)
60. Ysypo pere (Para el cáncer. Se machaca. En te o decocción)
61. Tapecue (Problems de estomago. Para lavar heridas se hierve)
62. Ybahai (Para diabetes. Se hierve y se toma 2-3 veces al día o como té)
63. Siempre viva (Para el corazón y calmar los nervios. Se hierve y se toma en mate)
64. Calabacita (Para diabetes, se hierve y toma como agua o en tereré)
65. Caaré (Antihelmítico. Se hierve y se toma en ayunas)
66. Chirca melosa (Diabetes. Se hierve y se toma como agua o té)
67. Kino kino (Para dolores reumáticos y para golpes. Se machaca y se hierve)
68. Capí-una (Para el riñon. En té o decocción)
69. Agrial (Para dolor de garganta. Se hace gárgara con agua fría)
70. Curupaymí (Para el reuma. Se toma en mate o tereré) Prophyllum lanieolatum (F)
71. Yerba mata (Para el corazón. Se toma con agua fría)

GRUPO I

1. Ambay
2. Sarandy Morotí
3. Piper sp No. 2 (Hojas lanceoladas)
4. Piper sp No. 1 (Hojas grandes anchas) (Yaguarundy)
5. Nangapiry (Eugenia uniflora)
6. Cedron-capii (Cymbopogon citratus)
7. Typycha-Kuratu (Scoparia dulsis)
8. Tape-cué (Acantos perum)
9. Koku (Allophillus edulis)
10. Colita (Cordia salicifolia)
11. Yvahai (Eugenia myrcianthes)
12. Paraparaí mí
13. Mil hombre
14. Marcela
15. Cola de caballo
16. Romero
17. Burrito
18. Jaguarundí
19. Alhucema
20. Espartillo guazú

Assay of Inhibitory Activity of Angiotensin Converting Enzyme (ACE)

1. Reagents

Hippuryl-L-histidyl-L-leucine (HHL)
Angiotensin converting enzyme (ACE)
DMSO
2% O-Phthalaldehyde methanol solution, freshly made (OPA)
0.3N HCl
3N HCl
Buffer A
Buffer B
Buffer C
MeOH

2. Buffer

Buffer A (pH 8.30)

(1) ---------- KH_2PO_4 0.34 g / 50 ml H_2O

(2) ---------- K_3PO_4 0.53 g / 50 ml H_2O

Buffer B (pH 8.30)

(1) ------ KH_2PO_4 2.04 g, NaCl 3.5 g / 100 ml H_2O

(2) ------ K_3PO_4 3.18 g, NaCl 3.5 g / 100 ml H_2O

Buffer C (pH 8.30)

(1) ------ KH_2PO_4 6.8 g, NaCl 8.77 g / 500 ml H_2O

(2) ------ K_3PO_4 10.61 g, NaCl 8.77 g / 500 ml H_2O

All buffer solutions are prepared by mixing of (1) and (2) concurrently, adjusted to pH 8.30

3. Preparation of ACE solution

1) homogenized in Buffer A at 4°C
2) centrifuge at 40000 G
3) stock a supernatant below 0°C

4. Assay method

HHL 0.1 ml
Sample 0.01 ml + ACE 0.14 ml incubation 37°C, 30 min.

+ NaOH + OPA 0.1 ml after 10 min. + HCl 0.2 ml

determination of fluorescence

1) prepared HHL (5 mM/1000 ml) 0.1 ml / tube
2) dissolve 0.01 ml of sample solution, add the ACE solution (30-40 μg protein / 0.14 ml) and start the enzyme reaction by incubation at 37°C for 30 min.
3) after the incubation add 0.3N NaOH to stop the reaction
4) add 0.1 ml of 2% OPA
5) after 10min., add 0.2 ml of 3N HCl and determined fluorescence (excitation wavelength at 340 nm and emission fluorescence wavelength at 455nm)
6) calculate the inhibition %

$$\text{Inhibition } \% = \frac{(A-B) - (C-D)}{A - B} \times 100$$

A; control C; sample
B; blank of control D; blank of sample

Cytotoxicity test against KB cell (*in vitro*)

1. Materials
2. Cell culture
3. Passage
4. Cell stock
5. Assay method

1. Materials

1. Preparation of reagents

PSS ---------- NaCl 8.5 g / Dist.H_2O 1000 ml

PBS ---------- NaCl 8.0 g
Na_2HPO_4 12 H_2O 2.9 g
KH_2PO_4 0.2 g
KCl 0.2 g
/ Dist.H_2O 1000ml

0.025% CV---------- crystal violet 9 H_2O 35mg
/ Dist.H_2O 100ml

0.02% EDTA -------- EDTA 44 mg / PBS 200 ml

10% $NaHCO_3$ -------- $NaHCO_3$ 10 g / Dist.H_2O 90ml

Eagles' MEM medium ----- Dried E. MEM 9.4 g
/ Dist.H_2O 1000 ml

Glutamine solution ----- Glutamine 2.92 g / E. MEM m. 100ml

Hanks solution. --------- Dried Hanks s. 9.8 g / Dist.H_2O 1000 ml.

0.25% Trypsin solution ----- Trypsin 0.5 g / PBS 200 ml
(is stirred over night at room temp.)

Complete MEM ---------- Glutamine solution 1.0 ml
10% $NaHCO_3$ 1.1 ml
FBS(fetal bovine serum)* 10.0 ml
E. MEM m.** ad. 100.0 ml
(aseptic manipulation in a clean bench)

* treated at 56°C for 30 min. and stocked at -25°C

Complete Hanks ----------	10% $NaHCO_3$	0.35 ml
	FBS*	2.0 ml
	Hanks s.***	ad. 100.0 ml

(aseptic manipulation in a clean bench)

* treated at 56°C for 30 min. and stocked at -25°C

2. Autoclaved sterilization

0.02% EDTA

10% $NaHCO_3$

Eagles' MEM medium**

Membrane filter set

Culture bottles

Test tube

Silicone rubber stoppers

Silicone rubber bulbs

Bottles with screw cap

Pastrur pipets

Dispenser pipets

Chips for dispenser pipet

3. Sterilization by membrane filter

Glutamine solution*

Hanks solution***

0.25% Trypsin solution

4. Dry heat sterilization

Pipets

2. Cell culture

1) storage cell was dissolved in warm water (37-42°C)
2) wash with 20 ml of complete Hanks solution in centrifuge tube by vibration.
3) centrifugate at 1200 rpm for 5 min.
4) pipette off the supernatant in a clean bench
5) inoculate in a culture bottle with complete MEM and incubate at 37°C

3. Passage

1) reverse the bottle cell cultured and decant the medium
2) add 5 ml of 0.02% EDTA and wash a cell sheet for 3 min
3) decant the EDTA solution and add 5 ml of 0.25% trypsin
4) after 1 min. hit the bottom of the bottle and scrap the cell sheet by pipetting and then vibrator .
5) the cell suspension was washed with 20 ml of complete Hanks solution
6) centrifugate at 1200 rpm for 5 min
7) pipette off the supernatant in clean bench
8) inoculate in a culture bottle with complete MEM and incubate at 37°C

4. Cell stock

1) the cultured cells are counted by blood cell counting plate under microscopy
2) dilute to 1-3 X 10^6 cells/ml with complete MEM containing 10% DMSO and 20% FBS*
3) after freezed at -20°C for 30 min., stock at -80°C in deep freezer

* treated at 56°C for 30 min. and stocked at -25°C

5. Assay method

I) cell-count and dilute to 2-3 X 10^4 cells/ml with complete MEM

2) prepare the cell suspension 3.9 ml (0.8-1.2 X 10^5 cells)/tube

3) add 0.1 ml of sample, dissolve in H_2O, EtOH or DMF, and Vibrate

4) incubate at 37°C for 72 hr. (tube angle at 10°)

5) after 72 hr. incubation, add 1 ml of 0.025% CV and incubate at 37°C for 15 min. (tube angle at 10°)

6) decant excess dye and add 10 ml of PPS

7) rotate the tube gently to wash cells

8) decant the PSS perfectly, and add 3 ml of 50% EtOH and then vibrate

9) determine OD (optical density) at 570 nm

IO) calculate the inhibition %

$$\text{Inhibition } \% = \frac{B - S}{B} \times 100$$

B; OD_{570} blank

S; OD_{570} sample

Cytotoxicity test against L5178Y cell (*in vitro*)

1. Materials
2. Cell culture
3. Passage
4. Cell stock
5. Assay method

1. Materials

1. Preparation of medium

Fischer's medium -------- Dried F. medium 10.5 g
Kanamycin sulfate 0.12 g
/ Dist. H_2O 1000 ml

Fischer's medium containing 10% Horse serum (HS) ----------
Fischer's medium 900 ml
Horse serum* 100 ml
(aseptic manipulation in a clean bench)

* treated at 56°C for 30 min. and stocked at -25°C

2. Autoclaved sterilization

Membrane filter set
Bottles with screw cap
Test tubes with screw cap
Pastrur pipets
Chips for dispenser pipet
Dispenser pipets
Centrifuge tubes with screw cap

3. Sterilization by membrane filter

Fischer's medium

4. Dry heat sterilization

Pipets

2. Cell curture

1) storage cells are dissolved in warm water (37 - 42°C)
2) wash with 20 ml of Fischer's medium in centrifuge tube by vibration.
3) centrifugate at 1200 rpm for 5 min.
4) Pipette off the supernatant in a clean bench
5) inoculate in a centrifuge tube with Fischer's medium containing 10% HS and incubate at 37°C

3. Passage

discribed as cell culture 3), 4) and 5)

4. Cell stock

1) The cultured cells are counted by coulter counter
2) dilute to 1-3 X 10^5 cells/ml with Fischer's medium containing 20% HS and 10% DMSO
3) after freezed at -20°C for 30 min, stock at -80°C in deep freezer.

5. Assay method

1) cells are counted by coulter counter and dilute to 5 X 10^4 cells/ml with Fischer's medium containing 10% HS
2) prepare the cell suspension 2.9 ml/tube
3) add 0.1 ml of sample solution (H_2O, EtOH, DMF or DMSO, 3%)
4) incubate at 37°C for 48 hr.
5) after incubate, the cells are counted by coulter counter
6) caluculate the inhibition %

$$\text{Inhibition } \% = \frac{A - C}{A - B} \times 100$$

(A : final cell count of blank, B : initial cell count of blank, C : final cell count of sample)

Ⅷ 植物化学での指導報告

富山医科薬科大学　薬学部助手
専門家　林　　利　光　報告

派遣期間　1985　9/10～11/ 9　2ヶ月
1986　4/10～10/14　6ヶ月

昭和60年度

1．パラグアイ共和国アスンシオン大学化学部に赴任中の業務内容（派遣期間9月10日～11月9日）

1－1　植物化学部門への機材の搬入、点検及び据付調整

薬草の熱時抽出を可能にするための機材
有機溶媒の蒸留を可能にするための機材
蒸留水製造用イオン交換樹脂、フィルター
} 等を携行し、植物化学研究室に搬入、点検した。

5月に搬入された機材の再点検を行うとともに、新たな携行機材の据付調整も実施した。

1－2　薬草の抽出エキスの調製条件の検討及びカウンターパートに対する技術指導

アスンシオン市内で購入した薬草の乾燥品を用い、70％EtOHで熱時抽出し、吸引濾過、減圧　濃縮及び凍結乾燥によるエキスの粉末化を試みた。

抽出条件：溶媒　70％EtOH（市販アルコールを蒸留したもの7に蒸留水3の割合に混合したもの）
試料　100～500 g（乾燥品）
時間　1時間　3回

対象試料：Cangorosa, Siete sangria, Cedron Paraguay, Siempre viva, Burrito, Marcera, Alhucema, Yaguarundy, Eucalipto, Parapara－i, Colita, Typycha Kuratu, Tapecue, Caa hee, Romero, Espartillo guazu, Colade caballo, Aromita, Chirca, Culantrillo, Piper sp. №3, Yvahai.

上記試料について　カウンターパート（Esteban Ferro, Lucia Franco, Cristna Theoduloz）と分担して抽出エキスを調製し、生物活性試験実施のため、日本へ郵送した。

1－3　抽出用薬草の目的地での採集及び写真、8㎜フイルム撮影（吉崎専門家と植物部門のカウンターパートに同行）

1回目：Paraguariでの採集（10月3日）
Mil hombre, Araticu－i, Yvahai, Cardo santo, Piper, Marcela.

2 回 目：Chaco で採集（10月27日～11月4日）

Kaatai, Altamisa, Kaare, Cabello de angel, Mandiyu－ra, Aguape－purua, Guayacan, Salvia, Verbena－i, Quebracho blanco, Paratodo, Caarurupe, Llanten de tierra, Taperyva－hu, Aguape, Cepa caballo, Pata de buey－i, Yvoty caaru.

1－4　アスンシオン第四市場での薬草の聞きとり調査（第四回目）

10月1日吉崎専門家及び Isabel Basualdo に同行。

154種の薬草を購入し整理、保存するようにした。

調査結果は資料1を参照。

1－5　植物化学部門でのセミナーの開催

2．パラグアイ薬草の抽出エキスについての生物活性試験

アスンシオン大学より送付されたパラグアイ薬草の抽出エキスについて、キサンチンオキシダーゼ（通風と関連がある）、β－グルクロニターゼ（肝性黄疸、結腸癌と関連がある）及びウレアーゼ（尿路結石と関連がある）に対する阻害作用を調べた。試験方法及び試験結果については資料2及び資料3に示した。

3．パラグアイ薬草の抽出エキスの分画及び活性成分の分離・精製

3－1　Romero のウレアーゼ阻害成分の分離・精製

Romero の抽出エキスについてウレアーゼ阻害活性を指標にしながら分画、成分の分離精製を実施中である。

3－2　Ñangapyry のキサンチンオキシダーゼ阻害成分の分離・精製及び構造決定

Ñangapyry の抽出エキスについてキサンチンオキシダーゼ阻害活性を指標にしながら、活性成分の分離・精製を行い、フラボノイドを二種単離した。これらの構造を各種スペクトルデータより検討し、各々 myricetin 及び myricitrin と同定した。このうち myricetin にキサンチンオキシダーゼ阻害作用が認められた。

3－3　Typychá kuratũ のβ－グルクロニダーゼ阻害成分の分離・精製

強いβ－グロクロニダーゼ阻害活性を示したTypychá kuratũの抽出エキスについて、活性成分を分離・精製中である。

4．生物活性試験の実施要領を8㎜フイルムに収録

61年度植物化学部門のカウンターパートへの技術移転の際の教材に使用するため、富山医科薬科大学薬学部生薬学研究室で実施している全ての生物活性試験について収録。

昭和61年度

1．アスンシオン大学化学部に赴任中の業務内容（派遣期間　4月15日～10月14日）

1－1　パラグアイの薬草利用者に対するアンケート調査

初年度にアスンシオン第四市場での薬草調査において認められた薬草について利用状況を明らかにする目的でアンケート調査を実試した。アンケート用紙を1,000部印刷・配布し、701名の回答を得た。薬草別利用者数、利用部位、服用方法、利用目的等の調査結果は、それぞれ資料4にまとめた。なお、回答者の内訳は資料5及び6の通りであった。

1－2　薬理部門に対する単独供与機材及び61年度携行機材の検収

1－3　植物化学部門のカウンターパートに対する酵素阻害実験の指導

キサンチンオキシダーゼ及びβ－グルクロニダーゼに対する阻害物質を検策出来るように各々の酵素阻害実験に関する8㎜フイルムを上映し、さらにセミナーで理解を深めさせ、具体的には、Lucia Franco と Cristina Theodunloz の二人のカウンターパートに対して技術指導を行った。

1－4　植物化学部門でのセミナーの開催

天然物化学の領域で薬草研究に関連のある文献を紹介するセミナーを四回開催した。

1－5　植物化学部門の研究室の整備

供与資機材を収納する戸棚を購入し、研究室内のガラス器具や試薬類を整理した。

1－6　抽出用薬草の自生地での採集

第一回目：San Loenzo で採集（9月26日）

Salvia, Molle－i

第二回目：Paragnari で採集（10月1日）

Yvope

1－7　薬草の抽出エキスの調整

Yvyra pyta, Molle－í, Cedron capii, Salvia, Chirca melosa, Typycha kuratũ, Romero, Burrito の抽出エキスを精製、日本へ送付。

1－8　薬草の写真撮影

アスンシオン大学構内、San lorenzo, Luque に生育している薬草を撮影した。また、アスンシオン第四市場で購入した薬草についても写真撮影を行った。

1－9　任国外出張

◎　アルゼンチンへの出張（6月7日～6月12日）

訪問先：ブエノスアイレス大学　　J. Coussio 教授

ラ・プラタ大学　　M. Najera 教授

ラ・プラタ自然科学博物館

◎　ブラジルへの出張（7月14日～7月19日）

訪問先：オズワルドクルス研究所　Dr. Jorge Bermudez

リオ・デ・ジャネイロ植物園

リオ・デ・ジャネイロ大学　Walter B. Mors 教授

サンパウロ植物園　Dr. Marcos Buckeridge

サンパウロ大学　Dr. M. Yoshida 及び Dr. M. Motidome

その他薬草研究家の中隅哲郎氏及び植物研究家の橋本梧郎氏と面談した。

◎ ボリビアへの出張（8月22日～8月26日）

訪問先：サンタ・クルスの市場

ラ・パスの市場

ラ・パスの自然治療医の診療所（Dr. T. T. Valencia）

その他ボリビア大学の農学部の教官たちと面談（Dr. F. K. Saucedo, Dr. J. magne, Dr. R. C. Staffer）した。

PLANTAS MEDICINALES UTILIZADAS EN EL PARAGUAY - ADQUIRIDAS EN EL MERCADO 4 -

1. Ñangapiry: para adelgazar y colesterol.
2. Typych-a curatú: para afecciones del hígado.
3. Agrial: para gárgaras en afecciones de la garganta.
4. Eucalipto: para combatir la tos y para inhalaciones.
5. Hinojo: para el estómago.
6. Chicoria: para combatir la tos y laxante.
7. Taropé: abortivo.
8. Llanten: para combatir la inflamación.
9. Orégano: para afecciones del estómago.
10. Menta: para afecciones del estómago.
11. Tupasy camby: para frialdad - afecciones femeninas.
12. Cardo santo (raíz): para dolores menstruales.
13. Apio Paraguay: refrescante.
14. Tatú ruguay: para apendicitis.
15. Tapecue: para afecciones de la piel.
16. Pynó guazú (raíz): antiinflamatorio, para golpes.
17. Molle-í: para afecciones de la garganta.
18. Mil hombre: abortivo - diurético. Aumenta la virilidad.
19. Yaguá rová: para el reumatismo.
20. Pata de buey: para afecciones del hígado y riñones.
21. Cedrón capií: para calmar los nervious y para el corazón.
22. Cangorosa: para úlceras y anticancerígeno.
23. Uruzú heé: para bronquitis.
24. Ysypó peré: para curar el cáncer.
25. Albahaca: para el estómago - flatulencias.
26. Arachichú: para fuego de San Antonio.
27. Yerba de lucero: para diarreas y afecciones estomacales.
28. Ruda: para purificar la sangre.
29. Poleo-í: para afecciones del estómago.
30. Malva de castilla: para palpitaciones del corazón.
31. Caarurupé: refrescante.
32. Tapecué: = 15.
33. Granada (fruto): para combatir diarreas.
34. Pacholí: para calmar los nervios.
35. Caraguatá rua: refrescante, corril.
36. Pata de buey-í: para afecciones del hígado y riñones.
37. Cocú: para afecciones del hígado.
38. Penicilina: para afecciones de la garganta.
39. Caá piky: refrescante.
40. Calaguala: para ácido úrico y el hígado.

41. Azafrán: para hepatitis.
42. Barba de choclo: para bajar la fiebre, refrescante.
43. Ysy: para bronquitis - cataplasma con sebo de buey.
44. Cabello de ángel: para el hígado.
45. Caraguatá (raíz): abortivo, corrial.
46. Toro ratí: para calmar la tos.
47. Caña brava: para el corazón.
48. Pyno-í: para purificar la sangre.
49. Mastuerzo: para el higado y los riñones.
50. Tetŭ caá: para el corazón.
51. Teyuyá: abortivo.
52. Altamisa-í: abortivo.
53. Canchalagua-i: abortivo, para regular la menstruación.
54. Ajenjo: contraceptivo.
55. Rábano: para limpieza del estómago.
56. Ruibarbo: abortivo - corrial.
57. Perdudilla negra: para hepatitis.
58. Rosa mosqueta: laxante.
59. Guayacán: para orina con sangre, y dolores.
60. Zarza mora: corrial, diurético.
61. Penacho (flor): para purificar la sangre.
62. Llanten de agua: para el hígado y para adelgazar.
63. Caatai: para hemorroides.
64. Mbaracayá nambí: para afecciones del hígado.
65. Caaré (planta entera): antiparasitario.
66. Cerraja: para úlceras.
67. Mango (flor): para calmar la tos - bronquitis.
68. Cardo santo (semilla): para asma en ahogos.
69. Terciopelo (flor): para el corazón en palpitaciones.
70. Capií catí: refrescante - corrial.
71. Ambay: para calmar la tos.
72. Yua pecá: para hemorragias.
73. Yva hai: para diabetes.
74. Yvyrá pytá (corteza): para afecciones de la garganta.
75. Ynga (corteza): para el colesterol y diabetes.
76. Curupica-y (corteza): para el hígado.
77. Aguapé puruâ: para hepatitis, y estómago inflamado.
78. Cocu (especie diferente ?): para afecciones del hígado.
79. Caraguatá (fruto): antiinflamatorio.
80. Rosa china (flor): para hemorragias.

81. Tamanda cuna: para combatir la sífilis.
82. Mbuy-say yú: para los riñones.
83. Cola de ratón: para el hígado.
84. Malva rapó piré: para inflamación.
85. Almique (fruto): para dolor de oído.
86. Siete sangría: para el corazón.
87. Urupevó: para hemorragias.
88. Quebracho blanco (corteza): para bajar la fiebre.
89. Urucú (semilla): para jaquecas.
90. Curatú (semilla): para afecciones del estómago - flatulencias.
91. Yvyrá tai (hoja); para el reumatismo.
92. Curupay-mí: para el reumatismo.
93. Yva hai-poñy: para combatir la diabetes.
94. Caavó tyrey: para el hígado - hepatitis.
95. Malva de olor; para el corazón y dolores de cabeza.
96. Alfalfa: diurético y flatulencias.
97. Sandia (semilla): para bajar la fiebre, antigripal.
98. Girasol (semilla): sin datos.
99. Boldo (hoja): para el estómago.
100. Guavirá (fruto): para purificar la sangre.
101. Caá heé (hoja): para diabetes.
102. Naranja dulce (cáscara del fruto):
103. Caygua-í (semilla): emético.
104. Yacaré yrupé: para el hígado y los riñones.
105. Culantrillo: para el hígado y los riñones.
106. Yatei caá: para el estómago.
107. Yerba mate: para el corazón.
108. Suico: para el estómago.
109. Menta-í: para el estómago y para los nervios.
110. Batatilla: refrescante.
111. Vira-vira: para el hígado.
112. Cambará: para calmar la tos.
113. Sidra (hoja): para los nervios.
114. Salvia né: para los dolores menstruales.
115. Yaguarundí: para calmar la tos.
116. Pindó (raíz): abortivo.
117. Sauco: para dolores de estómago.
118. Malva blanca (flor): para bronquitis y catarros.
119. Mbocayá (plaútula): para el reumatismo y abortivo.
120. Uruzú catí: antiparasitario.
121. Yerba buena: para el estómago.

122. Curuguai: para los riñones y el hígado.
123. Para-para'í: para las piedras en los riñones.
124. Zarzaparrilla: diurético.
125. Laurel de España: para el estómago.
126. Cumandá yvyrai: para calmar la tos.
127. Perdudilla blanca: refrescante, corrial.
128. Marcela: para diarreas y para el estómago.
129. Borraja (flor): para calmar la tos.
130. Romero: para adelgazar.
131. Yaguareté caá: para el estómago.
132. Ytá poty: para hemorragias.
133. Mbocayá (hojas): diurético.
134. Catuaba: afrodisiaco.
135. Manzanilla: para el estómago.
136. Cola de caballo: para el hígado y los riñones.
137. Espartillo-í: abortivo.
138. Paratodo (corteza): para diarreas, úlceras.
139. Tilo (flor): para calmar los nervios, para el corazón.
140. Cebada Paraguay: refrescante para bajar la fiebre.
141. Cepa caballo: diurético.
142. Ceibo (corteza): para hemorroides.
143. Mbaracayá nambí (especie diferente ?):
144. Burrito: para el estómago.
145. Sará (corteza): para diabetes.
146. Santa Lucía morotí (raíz):
147. Charrúa caá: para combatir diarreas.
148. Verbena: para dolores de garganta.
149. Caaré (semilla): antiparasitario.
150. Anís: para el estómago y flatulencias.
151. Siempre viva: para el corazón.
152. Eneldo (semilla): para el estómago.
153. Doradilla: para el hígado.
154. Cedrón Paraguay: para palpitaciones del corazón y para calmar los nervios.

Method to Determine Inhibitory Activity against Xanthine Oxidase

I. Reagents

substrate --- xanthine $C_5H_4N_4O_2$ (0.15 mM, 22.8 mg/L)*
enzyme --- xanthine oxidase (0.04 unit /ml, in phosphate buffer)
HCl, Na_2HPO_4, KH_2PO_4

2. Buffer --- 1/15M phosphate buffer (pH 7.5)

3. Procedure

1) put 1.0 ml of test solution into the test tube
2) add 2.9 ml of buffer solution
3) add 0.1 ml of enzyme solution
4) after preincubation at 25 ℃ for 15 min, add 2.0 ml of substrate solution
5) after incubation at 25 ℃ for 30 min, add 1.0 ml of HCl
6) determine the absorbance of the assay mixture at 290 nm

* dissolved in distilled water at about 60 ℃ by stirring for 2 - 3 hrs; prepare just before use

	sample	blank 1	control	blank 2
test solution (ml)	1.0	1.0	-	-
dist H_2O(ml)	-	-	1.0	1.0
buffer (ml)	2.9	3.0	2.9	3.0
enzyme (ml)	0.1	-	0.1	-
		preincubation at 25 ℃ for I5 min		
substrate (ml)	2.0	2.0	2.0	2.0
		incubation at 25 ℃ for 30 min		
IN HCl (ml)	1.0	1.0	1.0	1.0
		O.D. 290		

4. Calculation of the Inhibition %

$$\text{Inhibition } \% = \frac{(A-B)-(C-D)}{A-B} \times 100$$

A: optical density of control

B: optical density of blank 2

C: optical density of sample

D: optical density of blank 1

Method to Determine Inhibitory Activity against β-glucuronidase

1. Reagents

substrate --- p-nitrophenyl-β-D-glucuronide (0.1 M, 31.5 mg/ml)*
enzyme --- β-glucuronidase (from bovine liver, 15 units/g protein)**
AcOH, AcONa, HCl, tris(hydroxymethyl)aminomethane

2. Buffer --- 0.1M acetate buffer (pH 5.0)
0.01M tris-HCl buffer (pH 7.8)

3. Procedure

1) put 0.9 ml of test solution*** into the test tube
2) add 0.03 ml of substrate solution
3) add 0.1 ml of enzyme solution
4) after incubation at 37 °C for 30 min, add 0.25 ml of 0.2M Na_2CO_3
5) determine the absorbance of the assay mixture at 405 nm

* prepare just before use

** dissolve 20 mg of enzyme in 2 ml of 0.01 tris-HCl buffer (pH 7.8), centrifuge (10,000 rpm, 15 min) and use the supernatant as enzyme solution

*** test material is dissolved in 0.1M acetate buffer (pH 5.0)

	control	blank 1	sample	blank 2
acetate buffer (ml)	0.9	1.0	-	0.13
test solution (ml)	-	-	0.9	0.9
substrate (ml)	0.03	0.03	0.03	-
enzyme (ml)	0.1	-	0.1	-
	↓ incubation at 37 C for 30 min			
Na_2CO_3 (ml)	0.25	0.25	0.25	0.25
	↓ O.D. 405			

4. Calculation of Inhibition %

$E = A - B$

$S = C - (B + D)$

$$\text{Inhibition } \% = \frac{E - S}{E} \times 100$$

A: optical density of control

B: optical density of blank I

C: optical density of sample

D: optical density of blank 2

Method to Determine Inhibitory Activity against Urease

I. Reagents

Substrate --- Urea (3% urea in 0.1 M phosphate buffer, pH 6.7)

Enzyme --- Urease from Jack bean*

Indiacator --- Phenol red (0.1)**

2. Buffer --- 0.1 M Phosphate buffer (pH 7.7)

0.1 M Phosphate buffer (pH 6.7)

10 mM TEA (Triethanolamine) buffer (pH 7.0)

3. Procedure

I) put 0.25 ml of test solution into the test tube#

2) add 0.25 ml of enzyme solution

3) after incubation at 37 °C for 15 min, add 5.0 ml of substrate solution

4) measure the time of color change##

* Prepare the enzyme solution by dilution with TEA buffer in order to adjust the time of color change as 2 min in the case of blank

** Dissolve 0.1g of phenol red in 20 ml of ethanol and make up to 100 ml with distilled eater

\# Add 0.05 ml of 0.1% phenol red beforehand

\#\# The control solution is prepared with 5.5 ml of M phosphate buffer (pH 7.7) and 0.05 ml of 0.1% phenol red. Measure the time when the test solution shows same color as solution

	sample	blank
test solution (ml)	0.25	-
enzyme (ml)	0.25	0.25
distH_2O (ml)	-	0.25
	↓	incubation at 37 C for I5 min
phenol red (ml)	0.05	0.05
substrate (ml)	5.0	5.0
	↓	time

4. Calculation of the Inhibition %

$$\text{Inhibition \%} = \left(1 - \frac{\text{time of control}}{\text{time of sample}}\right) \times 100$$

キサンチンオキシダーゼ阻害活性

Table 1. X.O.－Inhibitory Activities of Extracts from Medicinal Plants in Paraguay

Name of Medicinal Plants		Inhibition % at 50μg/mℓ	Name of Medicinal Plants		Inhibition % at 50μg/mℓ
AMBAY	(r)	42.2	COLA DE CABALLO	(r)	20.4
SARA MOROTI	(h)	30.1	ROMERO P/F	(h)	49.6/69.4
	(r)	7.8		(r)	52.2(46)
PIPER SP, №2	(r)	6.1	BURRITO	(h)	28.3
PIPER SP, №1	(r)	53.2(46)		(r)	14.1
ÑANGAPIRY	(h)	74.7(6.6)	YAGUARUNDI	(h)	39.4
CEDRON-CAPII	(h)	32.9		(r)	44.9
	(r)	16.5	ALHUCEMA	(h)	45.2
TYPYCHA-KURATU	(h)	16.7		(r)	53.7(44)
	(r)	37.2	ESPARTILLO GUAZÚ	(h)	34.9
TAPE-CUÉ	(h)	25.9		(r)	37.6
KOKU	(h)	21.9	EUCALIPTO	(h)	52.2
COLITA	(h)	68.0(9.5)		(r)	59.8(35)
	(r)	57.8(36)	CANGOROSA	(h)	52.6
YVAHAI	(h)	75.3(2.5)	SIETE SANGRÍA	(h)	49.5
PARAPARAI MÍ	(h)	72.4	CEDRÓN PARAGUAY	(h)	45.5
	(r)	79.4(18)	SIEMPRE VIVA	(h)	48.8
MIL HOMBRE	(h)	13.0	KAÁ HEÉ	(h)	42.4
	(r)	0.3	AROMITA	(h)	77.3
MARCELA	(h)	93.9	CHIRCA MELOSA	(h)	54.3
	(r)	81.9(17)	CULANTRILLO	(h)	37.0
COLA DE CABALLO	(h)	59.8	PIPER SP, №3	(h)	19.2

( )＝IC_{50} (μg/mℓ)

r＝room temperature　　P＝Precipitate

h＝hot temperature　　F＝Filtrate

β－グルクロニダーゼ阻害活性

Table 2. β-Glucuronidase Inhibitory Activities of Extracts from Medicinal Prants in Paraguay

Name of Medicinal Plants		Inhibition % at 10μg/mℓ	Name of Medicinal Prants		Inhibition % at 10μg/mℓ
AMBAY	(r)	44.0(13.5)	ROMERO(precip)	(h)	35.5
SARA MOROTÍ	(h)	41.0	(filtrate)	(h)	61.9
	(r)	36.5(20.0)		(r)	30.0
PIPER SP, №2	(r)	24.0(23.0)	BURRITO	(h)	41.1
PIPER SP, №1	(r)	31.0(30.0)		(r)	24.5(30.0)
ÑANGAPIRY	(h)	56.5(6.0)	YAGURARUND	(h)	29.1
CEDRON-CAPII	(h)	21.0(42.0)		(r)	27.5(34.0)
	(r)	17.5(32.0)	ALHUCEMA	(h)	63.9
TYPYCHÁ-KURATÛ	(h)	79.5(5.4)		(r)	24.0(15.0)
	(r)	34.5(15.0)	ESPARTILLO GUAZÚ	(h)	59.6
TAPE-CUÉ	(h)	63.0(4.0)		(r)	36.3(17.0)
KOKU	(h)	76.5(5.0)	EUCALIPTO	(h)	76.8
COLITA	(h)	62.5(10.1)		(r)	40.1(21.0)
	(r)	56.0(5.0)	CANGOROSA	(h)	36.5
YVAHÁI	(h)	54.0(5.0)	SIETE SANGRÍA	(h)	46.4
PARAPARAI MÍ	(h)	56.2	CEDRÓN PARAGUAY	(h)	56.5
	(r)	67.0(6.4)	SIEMPRE VIVA	(h)	70.3
MIL HOMBLE	(h)	18.6	KAÁ-HEÉ	(h)	64.0
	(r)	16.0(72.0)	AROMITA	(h)	56.5
MARCELA	(h)	83.6	CHIRCA MELOSA	(h)	76.9
	(r)	32.0(16.5)	CULANTRILLO	(h)	48.9
COLA DE CABALLO	(h)	52.4	PIPER SP, №3	(h)	17.0
	(r)	14.5(30.0)			

( ) ; IC_{50} (μg/mℓ)

r ; room temperature

h ; hot temperature

ウレアーゼ阻害活性

Table 3. Inhibitory Activities of Extracts from Medicinal Plants against Urease in Paraguay

Maserial		Crude ext	n-Hexane ext	Insol	$CHCl_3$	H_2O ext	n-BuOH ext
AMBAY	r	33.2	–	–	–	–	–
SARA MOROTI	r	32.6	–	–	–	–	–
PIPER SP №2	r	19.1	–	–	–	–	–
PIPER SP №1	r	28.3	–	–	–	–	–
ÑANGAPIRY	r	47.4	31.5	39.7	23.0	–	70.4
CEDRON CAPÍI	r	11.0	–	–	–	–	–
	h	12.0	–	–	–	–	–
TYPYCHÁ-KURATÜ	r	29.2	–	–	–	–	–
	h	3.9	–	–	–	–	–
TAPE-CUÉ	r	9.2	–	–	–	–	–
KOKU	r	2.5	–	–	–	–	–
COLITA	r	35.2	–	–	–	–	–
	h	11.6	–	–	–	–	–
YVAHÁI	r	66.0	17.1	53.4	9.4	65.7	77.4
PARAPARAI-MÍ	h	32.1	–	–	–	–	–
MIL HOMBRE	h	6.9	–	–	–	–	–
MARCELA	r	32.9	–	–	–	–	–
	h	33.4	–	–	–	–	–
COLA DE CABALLO	r	33.2	–	–	–	–	–
	h	50.1	–	–	–	–	–
ROMERO	r	93.0	95.0	51.9	72.9	10.9	–
(precip)	h	95.0	–	–	–	–	–
(filtrate)	h	64.0	–	–	–	–	–
BURRITO	r	28.7	–	–	–	–	–
JAGUARUNDI	h	26.4	–	–	–	–	–
ALHUCEMA	h	18.7	–	–	–	–	–
ESPARTILLO GAUZU	h	8.8	–	–	–	–	–
EUCALIPTO	r	30.0	–	–	–	–	–
	h	40.8	–	–	–	–	–
CANGOROSA	h	62.1	–	–	–	–	–
SIETE SANGRIA	h	43.2	–	–	–	–	–
CEDRON PARAGUÁY	h	34.2	–	–	–	–	–
SIEMPRE VIVA	h	16.1	–	–	–	–	–
KAA HÉE	h	34.8	–	–	–	–	–
AROMITA	h	36.8	–	–	–	–	–
CHIRCA MELOSA	h	37.9	–	–	–	–	–
CULANTRILLO	h	32.2	–	–	–	–	–
PIPER SP. №3	h	31.0	–	–	–	–	–

Inhibitory Activity of Crude Extracts of Plants from PARAGUAY
(– Not Test, Inhibition % at 50μg/ml)

資料４　アンケート調査結果

RESULTADO DE LA ENCUESTA SOBRE PLANTAS MEDICINALES UTILIZADAS EN PARAGUAY

№	NOMBRE VULGAR	CANTIDAD	PARTE MAS UTILIZADA	OBJETIVO	MODO DE EMPLEO
1	AMBAY	354	Hoja	Tos – Catarro	Decoccion – Te – Mate
2	SARÁ MOROTÍ	23	Hoja – Corteza	Diabetes	Te
5	ÑANGAPIRY	210	Hoja	Hipertenssión – Diurético	Tereré – Decoccion – Mate
				Adelgazante	
6	CEDRÓN CAPII	209	Hoja	Estómago – Corazón	Te – Decoccion – Mate
				Tranquilizante	
7	TYPYCHÁ CURATÚ	135	Planta entera	Estómago – Digestivo	Te – Decoccion – Tereré
			Hoja	Estómago – Digestivo	Te – Tereré – Decoccion
8	TAPECUÉ	256	Hoja	Desinfectante – Cicatrizante	Lavaje – Te
			Planta entera	Desinfectante – Cicatrizante	Lavaje – Decoccion – Te
9	COCÚ	432	Hoja	Refrescante – Hepatitis –	Tereré – Te – Con agua
				Diurético	
11	YVAHAI	50	Hoja	Diabetes	Te – Decoccion – Mate
12	PARA-PARA-Í	165	Planta entera	Diurético–Rinón–Cálculo vesic.	Tereré – Te – Mate
			Hoja	Rinón–Cálculo vesic–Diurético.	Tereré – Te – Mate
13	MIL HOMBRE	206	Tallo	Diurético – Abortivo	Decoccion – Mate – Tereré
14	MARCELA	65	Flor y Hoja	Digestivo	Decoccion – Mate
			Hoja	Digestivo	Decoccion – Te
15	COLA DE CABALLO	146	Hoja	Refrescante – Diurético	Tereré – Te
			Hoja y Tallo	Refrescante	Tereré

№	NOMBRE VULGAR	CANTIDAD	PARTE MAS UTILIZADA	OBJETIVO	MODO DE EMOLEO
16	ROMERO	175	Hoja	Colesterol – Digestivo	Te
17	BURRITO	469	Hoja	Estómago – Digestivo	Te – Decocción – Mate
18	YAGUARUNDI	202	Hoja	Tos – Catarro	Te – Decocción
19	ALHUCEMA	24	Hoja	Estómago – Reumatismo	Te – Decocción
20	ESPARTILLO GUAZÚ	16	Planta entera	Dolor de muela	Decocción
21	EUCALIPTO	350	Hoja	Expectorante – Tos	Te – Inhalación – Decocción
22	CANGOROSA	329	Hoja	Cicatrizante–Abortivo–Cáncer	Te – Mate
			Hoja y Raíz	Cicatrizante	Decoccion – Mate
23	SIETE SANGRÍA	112	Planta entera	Hipertensión – Corazón	Mate – Te
			Hoja	Hipertensión	Te
24	CEDRÓN PARAGUAY	230	Hoja	Corazón – Sedante	Te – Mate
25	SIEMPRE VIVA	78	Flor	Corazón – Sedante	Mate – Te
26	CAÁ HEÉ	195	Hoja	Diabetes	Te – Mate – Decocción
27	AROMITA	116	Flor	Corazón	Decocción – Te
28	CHIRCA MELOSA	26	Hoja	Estómago	Decocción
29	CULANTRILLO	95	Hoja	Refrescante	Terere – Decocción
31	CAMBARÁ	107	Hoja	Tos	Te – Decocción
32	PARATODO (PIRÉ)	24	Corteza	Herida – Sarna – Refrescante	Mate – Lavaje – Te
34	ALTAMISA ITÉ	98	Hoja	Dolor de cabeza	Directamente – Te – Decocción
35	CABELLO DE ANGEL	127	Cabello	Diurético – Infec. vías urinarias	Te – Mate

Nº	NOMBRE VULGAR	CANTIDAD	PARTE MAS UTILIZADA	OBJETIVO	MODO DE EMPLEO
36	CAATAI	96	Hoja	Sarna	Uso externo – Decocción
			Planta entera	Sarna – Desinfectante	Lavaje
37	CAARÉ	189	Hoja	Vermífugo	Decocción – Te
			Hoja	Vermífugo	Decocción – Te
38	SAUCO	91	Hoja	Hepatitis	Te
39	MOLLE-Í	67	Hoja	Garganta	Gárgaras – Te
			Hoja y Raíz	Garganta	Gárgaras – Te
40	SALVIA	303	Hoja	Antiespasmódico – Digestivo	Te – Mate – Decocción
41	CARAGUATA RUA	86	Hoja	Refrescante	Tereré
42	PINDÓ (RADÓ)	35	Raíz	Abortivo – Diurético	Te – Decocción
43	YSY	19	Gomorresina	Piel – Bronquitis	Friccionar
44	GUAYACÁN	56	Corteza	Diarrea	Decocción
45	PERDUDILLA NEGRA	51	Planta entera	Refrescante	Tereré – Mate
			Raíz	Refrescante	Tereré – Mate
46	MALVA BLANCA	331	Hoja y Flor	Tos – Catarro	Decocción – Te
			Hoja	Tos – Catarro	Decocción – Te
47	GUAYABA	326	Hoja	Garganta – Diarrea	Decocción – Gárgaras – Te
48	TAPERYVÁ-HÚ	94	Raíz	Vermífugo	Te – Decocción
49	PENICILINA	108	Hoja	Antiséptico	Lavaje
50	VERBENA-Í	277	Hoja	Depurativo – Digestivo	Te – Decocción
				Garganta	

Nº	NOMBRE VULGAR	CANTIDAD	PARTE MAS UTILIZADA	OBJETIVO	MODO DE EMPLEO
51	TAMANDÁ – Í	3	Fruto	Asma – Bronquitis	Decocción
			Corteza	Asma – Expectorante	Decocción
52	AGUAPÉ PURUÁ	173	Hoja	Hepatitis – Refrescante	Tereré – Con agua
			Planta entera	Hepatitis – Refrescante	Tereré – Decocción – Con agua
53	YVYRA PYTA	23	Gorteza	Garganta – Desinfectante	Te – Gárgaras
54	ALCANFOR DEL CAMPO	48	Hoja	Estómago – Catarro	Te – Decocción
55	CALAGUALA	135	Hoja	Abortivo – Amenorrea	Te – Decocción
56	YVOPE	85	Fruto	Anticaspa	Lavaje
			Semilla	Anticaspa	Lavaje
57	CARDO SANTO	99	Raíz	Depurativo – Digestivo	Decocción – Tereré
			Hoja	Dolor de vientre	Decocción – Te
58	PYNÓ GUAZÚ	50	Hoja	Refrescante	Tereré
			Planta entera	Depurativo	Te
59	TAYUYÁ	19	Raíz	Diurético	Tereré – Decocción
60	CAÑA BRAVA	88	Raíz	Diurético – Reumatismo Refrescante	Tereré – Te – Decocción
61	TAROPÉ	103	Raíz	Refrescante	Tereré
			Planta entera	Refrescante	Tereré
62	YAGUÁ ROVA	71	Raíz	Abortivo – Reumatismo	Te – Decocción
63	MOLLE	68	Hoja	Garganta	Buche – Gárgaras – Decocción
			Raíz	Garganta	Gárgaras – Decocción
			Hoja y Tallo	Garganta	Gárgaras – Decocción

資料5　回答者の内訳（居住地）

RESULTADO DE LA ENCUESTA SOBRE PLANTAS MEDICINALES UTILIZADAS EN PARAGUAY

Iguazú	12
Asunción	251
Lambaré	14
San Lorenzo	111
La Colmena	7
Areguá	6
Caacupé	6
Minas Cué	7
Isla Pucú	2
Itacurubí de la Cordillera	15
Itaguá	6
Fernando de la Mora	20
Paraguarí	5
San Bernardino	2
Ipacaraí	1
Luque	49
Capiatá	82
Villeta	4
Quiindy	4
Yaguarón	3
Itá	11
Tobatí	9
Ñemby	7
Carapeguá	4
San Juan Bautista (Misiones)	1
San José de los Arroyos	1
Caaguazú	1
Villa Hayes	1
Mcal. Estigarribia (Chaco)	1
Villa Elisa	2
Mariano Roque Alonso	2
Ypané	1
Mayor Martínez	1
Ayolas	7
Encarnación	11
Pirapó	5
Zeballos Cué	2
Lugar sin nombre	27
TOTAL	701

資料6. 回答者の内訳（性別、年令）

RESULTADO DE LA ENCUESTA SOBRE PLANTAS MEDICINALES UTILIZADAS EN PARAGUAY

Items of answers

Age	Male	Female
0 – 20	4	16
20 – 29	30	71
30 – 39	51	88
40 – 49	41	127
50 – 59	40	104
60 – 69	22	48
70 – 79	9	17
80 – 89	1	6
90 – 99	–	1
Uncertain	7	18
Total	205	496
Average of age	44.0	44.1

Ⅸ 薬理学での指導報告

富山医科薬科大学医学部薬理学講座
教 授 中 西 穎 央
指導 助教授 武 田 龍 司
助 手 百 瀬 弥寿徳、 助手 山 崎 弘 美

D. Ibarrola が将来パラグアイ国において種々の薬用植物の薬効について、薬理学的研究を達成することを目標として、薬理学講義ならびに実習項目を設定した。1986・5・13より1986・12・25の間下記の項目について薬理学研究室において研修した。

Ⅰ．生化学薬理領域

1．動物組織の細胞分画法

マウスおよびラットの肝と脳について細胞下分画法を習得した。

2．脳および肝細胞下画分に局在する酵素活性の測定

可溶画分のADH、可溶画分、ミトコンドリア画分、ミクロゾーム画分のALDH活性の測定法を習得し、これら酵素活性に及ぼす薬物 in vivo 処置の影響を検討した。

3．肝細胞下画分のALDHについて、カイネティックパラメーター（Km値，Vmax値）の測定法を研修した。

4．SDS－ポリアクリルアミドゲル電気泳動法の習得。

Cytochrome P－450多型について研修した。

5．細胞分離法

肝細胞分離法の習得

肝の解剖学を講義し、肝細胞を中心静脈域と門脈域と分けて分離する技術を習得した。

Ⅱ．生理薬理学領域

1．イヌを用いる実習

(a) イヌの静脈注射法，腹腔内注射法

イヌの呼吸曲線、血圧変動の記録を研修し、代表的薬物の呼吸・血圧に及ぼす影響を研修した。

(b) 腹膜外尿管露出法による利尿実験

イヌ尿管を腹膜外に露出してカニューレを挿入し利尿作用の研究を行った。

高張塩，臨床的に用いられている種々の利尿剤について利尿作用を調べた。

2．モルモットを用いる実習

摘出腸管運動に及ぼす薬物作用を実習した。

3．マウスを用いる実習

マウスを用いLD　50の測定を行った。硝酸ストリキニンを腹腔内投与してLD　50を算出した。Up and Down 法を用いる。

4．ウサギを用いる実習

ウサギの呼吸・血圧に及ぼす薬物作用、摘出腸管運動に及ぼす薬物作用を研修した。

5．ガマ摘出心臓についての実習

八木法によりガマ摘出心臓を灌流し、薬物作用を調べた。

学外研修

1．信州大学医学部薬理学教室（千葉茂俊教授）において1週間、心臓・血管系（イヌ）の薬理学実験を見学した。

2．日本ベーリンガー川西医薬研究所（薬理部長　公平宏博士）において一般薬理実験及び毒性実験を見学した。

X　C/P　日本での研修報告

C/P Dr. Isabel Basualdoの研修報告

Report on Training of C/P Isabel Basualdo in Japan

Herbal Garden, Faculty of Pharmaceutical Sciences
Toyama Medical and Pharmaceutical University
Period: from May 11 to August 2, 1986

This training aims at studying morphology of crude drugs, putting Paraguayan herbs in order, cultivating herbs, and straightening and maintaining a herb garden.

In the practice of the training, the cooperation by Suzuki, assistant, and technical personnel of the garden was devoted.

Paraguayan materials which were brought by Isabel was too much damaged to be used, so c/p received the training (Suzuki was in charge) on processes of morphological study with specimens in this garden. In regard with putting Paraguayan species in order, a part of those which were surveyed and collected in Paraguay was sticked to board to be placed in order, and botanical names as well as common names were classified on some of these species.

Regarding cultivation, the guidance on cuttage practice was conducted by technical personnel. Besides, c/p observed and studied procedures practised in this garden.

Concerning the straightening and maintenance of a herb garden, the constitution of our garden was so completely explained that it could become a good reference in maintaining a botanical garden in Asuncion University, and essential matters in practice were guided. In addition, following places were visited to grasp the functions. Herb Cultivation and Guidance Center of Toyama Prefecture, Medicinal Plants Garden of Kyoto Pharmaceutical University, the Kyoto Botanical Garden, Kyoto Herbal Garden, Pharmacognosy Laboratories, Central Research Division, Takeda Chemical Industries, Ltd., Sugadaira Herb Garden of Nagano Prefecture, Kitamimaki Experiment Land of Nagano Prefecture, etc., were visited, and the practice of distinguishing was conducted.

We convince, from above technical experiences, c/p has sufficiently acquired the outline of the technique required for the study of herbs. Owing to the cooperation of Suzuki, assistant, and technical personnel, discussion was lastly held relating botany in Asuncion University. Then the content of the discussion was straightened, and the sentence was made to determine the purport of further cooperation in study in the future, which was mutually understood.

C/P Miss Nelida Soria の研修報告書

REPORT OF THE TRAINING IN THE MEDICAL AND PHARMACEUTICAL UNIVERSITY OF TOYAMA

Instructer: Dr. Shoichi Suzuki

1) Use of electroforesis to detect morfological variation

We have analysed 4 species of trichosanthes (Flia. cucurbitaceae), Datura (Flia. Solanaceae) and Cassia (Flia. Leguminosae).
We have worked with leaf, root and seed (0,5 grs.), prepared the sample of prottein concentration by Extracted them mixed in TRIS, and Polyvinil pyrrolidine at PH 6,8.
The Polyacrilamide Gels was used in final concentration of 12,5 %.
The sample was loaded onto de gel (about 60 mc.) and the electroforesis was continued until the tracking was migrated nearly to the gel bottom.
We used 50 volt during 2 hours and them have changed to 170 volt. Finally the Gel was stain in cromassie blue during 45 minutes after this, was destain in mixed of Metanol, Acetic acid and water.
The Stainded Protein bands was photographed before dry it.
The Protein banding patter was analysed with a computer, using a Program colled "Multi variant analysis".
We could not get a good result because took a long time before we can use correctly the technique.

2) Determination of the botanical origins of cruds drugs

The method is colled S.U.M.P. (Suzuki's Universal Macro Printing).
We printed the leaves of Cassia and have observated it at the microscopy, then we took picture, develoment and printed on paper. We could analyses the hair and the cell of the leaves.

3) Studies on propagation method for cultivation

For the cuttage test has used 60 species of the medicinal plants from the garden.
The samples were cutted and putting into water during one day before to plant then.

Work outside

8 - 8 - 85 - Went to togamura

8 - 23 - 85 - Went to tateyama mountain tc study the different between plants from cold and hot place.

8 - 30 - 85 - Visited the Pharmaceutical company in Toyama.

9 - 4 - 85 - Visited the Kyoto Botanical Garden.

9 - 5 - 85
9 - 6 - 85 - Attended to meeting of Medical and Pharmaceutical Society for wakan Yoku in Kyoto.

9 - 20 - 85 - Visited the Medicinal plants research Center of Toyama Prefecture.

NOTE: This repot is already incluyed in the ferst report of the Project.

Selvia Soria

phytochemistry から C/P として 1986. 6/23 ～ 8/22 まで研修の
Dr. Esteban Ferro の報告書

LABORATORY REPORT. DEPARTMENT OF PHARMACOGNOSY . MEDICAL AND PHARMACEUTICAL UNIVERSITY. TOYAMA.

BY: DR. ESTEBAN A. FERRO BERTOLOTTO
FROM: JUNE,23,1986
TO : JULY,22,1986

Along this month were performed the following activities:
-Use of Hitachi 220 U.V. spectrophotometer.
-Preparation of the enzyme Aldose Reductase(AR) from rat lenses for inhibition assays.
-AR assay fallowing the time course using water and DMSO.
-AR assay using diferent solvents.
-AR assay using plant extracts and pure compounds to measure the inhibition percent and calculate the IC_{50} of the active samples.
-Checking of the AR enzyme activity.
-Preparation of urease from Canavalia ensiformis DC (Jack bean).
-Solvent fractionation of a crude extract of Marcela and further AR inhibition test of each fraction.
-Bibliographic search of the genus Citharexylum(Sara moroti).

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PREPARATION OF AR CRUDE ENZYME FROM RAT LENSES

Rats(Wistar strain) weighting 200-300 g. were put in a glass jar with ether.Then they were killed by broking their necks and the eye lenses were extracted using scissors . The lenses were put in a phosphate buffer solution 0.1 M,pH: 6.8 containig 1 mM of 2-mercaptoethanol and 1 mM of NADP. This solution was kept in a ice bath, using 0.1 ml of solution per lens, and was stored frozen at -25° C until the enzyme preparation. The lenses with the buffer solution were melted using an ice bath, and then tranfered to an tissue homogeneizer and stirred till complete lenses disruption and milky aspect of the mixture.This mixture were put into cool certrifuge tubes and centrifuged 15 min.at 4° C at 12000 r.p.m.(10000 g.). The supernatant contains the crude AR enzyme and was transfered to vials for further assays. The enzymatic

activity was tested using the assay conditions, phosphate buffer 0.1 M, pH 6.2, NADPH, with and without the substrate (+ glyceraldehyde) and measuring the absobance decay at 340 nm. The enzyme was diluted using phosphate buffer 0.1M pH 6.8 to get an absorbance decay of 70% in 200 seconds. The crude enzyme was separeted in aliquots of 1.0 , 1.3 , 1.5 and 1.8 ml. and stored at -78° C.

AR ASSAY

The AR assay was performed using a phosphate buffer solution 0.1 M, pH 6.2 containing NADPH 0.104 mM. A part of this solution (Substrate +) was used for disolving the substrate, + - glyreraldehyde 10 mM. All the assays were performed fallowing this scheme:

	CONTROL	BLANK-1	SAMPLE	BLANK-2
PO_4 buffer (ml)	0.97 (+)	0.97 (-)	0.97 (+)	0.97 (-)
Sample (ml)	-	-	0.01	0.01
Water or DMSO (ml)	0.01	0.01	-	-
AR crude enz. (ml)	0.02	0.02	0.02	0.02

The assay was performed at 25° C, keeping the buffer solution in a water bath and sirring every 5 min. The solutions were transfered using dispensors to 1 ml. UV cells, and sirring with glass rods after adding the reagents. The enzyme was added and mixed 40 seconds before starting the scan. Unless other conditions be reported the assays were plotted using a 220 Hitachi VIS/UV spectrophotometer at 340 nm, slit 2, response 4, time drive 60 mm/min. and scale 0.00-- 0.05. Two sets of cells were used and always in the same way and position.

AR TIME-COURSE USING WATER (CONTROL)

TIME (minutes)	ABSORBANCE	TIME (minutes)	ABSORBANCE
2	0.0009	20	0.182
6	0.058	26	0.222
10	0.094	30	0.244
12	0.140	36	0.269
14	0.132	40	0.281
16	0.150	44	0.290
18	0.157	50	0.297

This data were plotted giving a linear relationship till 10-14 minutes after adding the enzyme.

AR ASSAY USING DIFERENT SOLVENTS

In the usual conditions of the AR assay were tested diferent solvents during 300 seconds and noted the effect in the absorbance decay.

SOLVENT	ABS.DECAY AT 200"	ABS.DECAY AT 300"
Water	44 %	67%
DMSO	44.2%	67%
Ethanol	41%	61%
Propilenglycol	10.5%	16.3%
Methanol	43%	63.3%

The propilenglycol exibits a strong inhibitory effect on AR.The others solvents have a very close response among them.

AR ASSAY USING PLANT EXTRACTS AND PURE COMPOUNDS. MEASURING OF INHIBITION % AND IC_{50}.

Using the AR assay conditions noted previously were tested several crude extracts of medicinal plants of Paraguay. Also were checked isolated compounds,fractions and a reference(quercitrin). In each batch is noted the inhibition percent of each sample,the initial and final value of the quercitrin and the IC_{50} of the samples with strong inhibitory effect. The samples that exibited inhibition % more than 50 were repeated and the data showed is an average of this results. The inhibition percent was calculated using a control line,obtained plotting the variation of absorbance of DMSO along the assay. Blank control was performed till get a stable condition(about 40-70 minutes after disolving NADPH)and every 4 samples. This blank line shows a constant slope till 230-260 minutes after disolution of NADPH. So the useful time for assay is arround 3 hours.

For the calculation of inhibition % was used the formula:

$$\text{Inhibition \%} = \frac{\text{Absorbance Control} - \text{Absorbance Sample}}{\text{Absorbance Control}} \cdot 100$$

or Inhibition % = $\frac{(\text{CONTROL - BLANK 1}) - (\text{SAMPLE - BLANK 2})}{(\text{CONTROL - BLANK 1})} \cdot 100$

The samples of crude extracts were tested using a concertration of 1×10^{-5} g/ml (10 micro g/ml). For the calculation of IC_{50} were tested several concentrations fron 5×10^{-5} to 5×10^{-7} g/ml and plotted the inhibition % againt the log of the concentrations. The concentration that exibit 50% inhibition corresponds to the IC_{50}. This value is expresed in g/ml for crude extracts and fractions and using moles/l for pure compounds.

July,3.1986

AR Lot#3

SAMPLE	INHIBITION %
Quercitrin $1x10^{-6}$ g/ml	56.6
Quercitrin $5x10^{-6}$ g/ml	68.3
Quercitrin $1x10^{-5}$ g/ml	87.2
Propilenglycol(0.01ml)	68.8
Sará Morotí Bark($5x10^{-6}$ g/ml)	40.3
Kaá-Heé	23.8
PAR 2+6($5x10^{-6}$ g/ml)	34.7
Sará Morotí Leaves($5x10^{-6}$ g/ml)	61.7

IC_{50} of quercitrin: $4.6X10^{-7}$ g/ml

July,7,1986

AR Lot#3

SAMPLE	INHIBITION%
Quercitrin $1x10^{-5}$ g/ml	76.3
Quercitrin $5x10^{-6}$ g/ml	72.3
Quercitrin $1x10^{-6}$ g/ml	47.2
Quercitrin $5x10^{-7}$ g/ml	26.7
Quercitrin $1x10^{-7}$ g/ml	16.5
Cambará	85.4
Araticuí	31.0
Altamisa-ité	36.3
Cabello de Angel	35.0
Caa-tai	20.1
Caa-rẽ(roots)	8.2

SAMPLE	INHIBITON%
Caa-rê(aerial parts)	9.2

IC_{50} quercitrin=$1x10^{-6}$ g/ml ----- 2.45×10^{-6} M

July,9,1986

AR Lot#3

SAMPLE(g/ml)	INHIBITION %
Quercitrin $1x10^{-6}$	50.4
Cambará $1x10^{-5}$	85.7
Cambará $5x10^{-6}$	56.2
Cambará $1x10^{-6}$	36.4
Cambará $5x10^{-7}$	16.5
Cambará $1x10^{-7}$	19.0
Sauco	28.2
Molle-í	55.1
Salvia	50.4
Caraguata-ruá	23.8
Pindó	16.4
Ysy(leaves)	48.6
Guayacán(bark)	79.9
Quercitrin $1x10^{-6}$	24.2

IC_{50} Cambara:3×10^{-6} g/ml

July,11,1986

AR Lot#3

SAMPLE	INHIBITION %
Quercitrin $1x10^{-6}$	47.4
Perdudilla Negra	15.0
Malva Blanca	30.6
Guayaba	54.4
Taperyva-hu	28.2
Penicilina	26.6
Verbena-í	49.2

SAMPLE	INHIBITION%
Aguape-puruá(roots)	14.6
Aguape-puruá(aerial parts)	21.1
Yvyra-pytá	11.2
Quercitrin $1x10^{-6}$	32.7

July,12,1986
AR Lot#3

SAMPLE	INHIBITION %
Quercitrin $1x10^{-6}$	70.7
Alcanfor(de hoja)	81.1
Calahula	29.9
Ybopé(Gleditsia sp.)	34.4
Paratodo(leaves)	44.7
Sará morotí bark(E.Ferro)	51.3
S.Morotí bark(E.Ferro)precipitate	53.7
S.Morotí bark(E.Ferro)n-BuOH	77.7
S.Morotí bark(E.Ferro)water	30.2
Quercitrin $1x10^{-6}$	67.0

July,15,1986
AR Lot#3

SAMPLE(g/ml)	INHIBITION %
Quercitrin $1x10^{-6}$	61.0
S.Morotíleaves E(Horie)$5x10^{-6}$	66.0
S.Morotí 1(Horie)	43.8
S.Morotí 2(Horie)	33.1
S.Morotí 3(Horie)	78.7
S.Morotí 4(Horie)	84.3
S.Morotí 1 (6) (Horie)	63.2
S.Morotí 3 (6) (Horie)	6.1
Molle-í $5x10^{-5}$	86.7
Molle-í $1x10^{-5}$	54.0
Molle-í $5x10^{-6}$	18.4
Molle-í $1x10^{-6}$	17.2
Molle-í $5x10^{-7}$	1.0

SAMPLE	INHIBITION %
Salvia $5x10^{-7}$	0.0
Salvia $1x10^{-6}$	6.3
Salvia $5x10^{-6}$	14.1
Salvia $1x10^{-5}$	39.4
Salvia $5x10^{-5}$	78.0
Ysy $5x10^{-7}$	0.0
Ysy 1x10-^{6}	7.0
Ysy $5x10^{-6}$	14.9
Ysy $1x10^{-5}$	57.9
Ysy $5x10^{-5}$	94.1
Quercitrin $1x10^{-6}$	61.5

July,16,1986
AR Lot#3

SAMPLE	INHIBITION %
Quercitrin $1x10^{-6}$	71.3
Guayacán(bark)$5x10^{-7}$	10.3
Guayacán(bark)$1x10^{-6}$	17.4
Guayacán(bark)$5x10^{-6}$	53.1
Guayacán(bark)$1x10^{-5}$	85.5
Guayaba $5x10^{-7}$	7.4
Guayaba $1x10^{-6}$	0.0 (?)
Güayaba $5x10^{-6}$	22.5
Guayaba $1x10^{-5}$	65.3
Guayaba $5x10^{-5}$	81.8
Verbena-í $1x10^{-6}$	3.1
Verbena-í $5x10^{-6}$	27.0
Verbena-í $1x10^{-5}$	61.0
Verbena-í $5x10^{-5}$	92.0
Alcanfor(dehoja)$5x10^{-7}$	0.0
Alcanfor(de hoja)$1x10^{-6}$	17.1
Alcanfor(de hoja)$5x10^{-6}$	35.6
Alcanfor(de hoja)$1x10^{-5}$	67.3
Alcanfor(de hoja)$5x10^{-5}$	90.8
Quercitrin 1x10^{-}6	45.2

IC_{50} Guayacán (bark)= 4.3×10^{-6} g/ml

IC_{50} Guayaba = 7.8×10^{-6} g/ml

IC_{50} Verbena-í = 8.0×10^{-6} g/ml

IC_{50} Alcanfor(de hoja) = 7.0×10^{-6} g/ml

July,17,1986

AR Lot#3

SAMPLE (g/ml)	INHIBITION %
Quercitrin $1x10^{-6}$	68.0
S.Morotí bark(E.Ferro) $1x10^{-6}$	1.5
S.Morotí bark(E.Ferro) $5x10^{-6}$	15.4
S.Morotí bark(E.Ferro) $1x10^{-5}$	35.0
S.Morotí bark(E.Ferro) $5x10^{-5}$	84.0
S.Morotí bark(EF)n-BuOH $1x10^{-6}$	2.6
S.Morotí bark(EF)n-BuOH $5x10^{-6}$	29.6
S.Morotí bark(EF)n-BuOH $1x10^{-5}$	71.2
S.Morotí bark(EF)n-BuOH $5x10^{-5}$	94.7
PAR 1(Horie)$5x10^{-7}$	82.6
PAR 2 (Horie)$5x10^{-7}$	34.9
PAR 3m(Horie)$5x10^{-7}$	44.2
PAR 3a(Horie)$5x10^{-7}$	0.18
PAR 4(Horie) $5x10^{-7}$	7.2
PAR 5(Horie) $5x10^{-7}$	0.0(?)
PAR 6(Horie) $5x10^{-7}$	29.3
Quercitrin $1x10^{-6}$	60.0

IC_{50} Sará Morotí Bark(E.Ferro) Crude extract=1.6×10^{-5} g/ml

IC_{50} Sará Morotí Bark(E.Ferro) n-BuOH = 7.0×10^{-6} g/ml

July,19,1986
AR Lot#3

SAMPLE(g/ml)	INHIBITION %
Quercitrin $1x10^{-6}$	70.0
Marcela A $1x10^{-5}$	83.0
Marcela B $1x10^{-5}$	31.8
Marcela C $1x10^{-5}$	58.9
Marcela E $1x10^{-5}$	85.8
Marcela F $1x10^{-5}$	66.5
S.Morotibark (EF)$1x10^{-5}$	40.9
S.Moroti bark(EF)$5x10^{-5}$	81.2
S.Moroti bark(EF)BuOH $1x10^{-5}$	70.7
S.Moroti bark(Horie)$1x10^{-5}$	69.0
S.Moroti bark(Horie)$5x10^{-5}$	96.5
Quercitrin $1x10^{-6}$	64.7

The strongest inhibitory activity of Marcela fractions was found in the n-BuOH fraction(E). This fraction will be processed for the isolation of the active compounds.
The comparison of two extrcts of Sara Moroti bark at hot conditions show different activities. Both samples were collected in the same season in different ,but close,pleaces. TLC compairson should be done with both extracts.

CHECKING OF THE AR ENZYME ACTIVITY

Was calculated using the formumla:

$A = \varepsilon \cdot b \cdot c$

A: absorbance of the control at the middle stable control line. This value was calculated using several control lines of the same enzyme lot.(#3).The mean value was corrected to 60 seconds.

ε: $\varepsilon_{NADPH} = 6.22 \times 10^{6}$ cm^2/ mol

b: path length = 1 cm

c: concentration of NADPH.

The A value in the assay conditions was: 0.05 $\pm$ 0.0014 using a scale value of 0.05. The c value in the formula gives the rate of comsuption of NADPH and the enzyme activity when the A value is corrected for 60 seconds. With these data the AR activity is : 1.4.

PREPARATION OF UREASE FROM CANAVALIA ENSIFORMIS SEEDS

200 g. of powder of C.ensiformis DC (seeds) and 3 spoons of Hyflo Super Cell were extracted 4-5 min. at 0-5° C(ice bath) with 1 l. of acetone 30%(aqueos) containing 8-10 ml of 2-mercaptoethanol and 1 mM of Na_2EDTA. The suspension was filtered in vacuo at 0-5° C during 15-25 min.(optimun 15 min) To the clear solution was added cold acetone dropwise, 170 ml in a cool room,and the mixture was kept in this conditions over nigth. After decanting the supernatant the precipitate was centrifuged 10 min. at 3000 r.p.m and 4° C. The precipitate was dissolved using 0.1M phosphate buffer pH 6.7 containing 0.1 M of triethanolamine(TEA),15 ml. This mixture was centrifuged 20 min. at 12000 r.p.m. and 4° C. The supernatant contains the urease and is stored at 5° C. The precipitate of the last process can be dissolved ajn using the PO_4-TEA buffer to get more enzyme. The activity of the enzyme was measured using assay conditions and the fallowing formula:

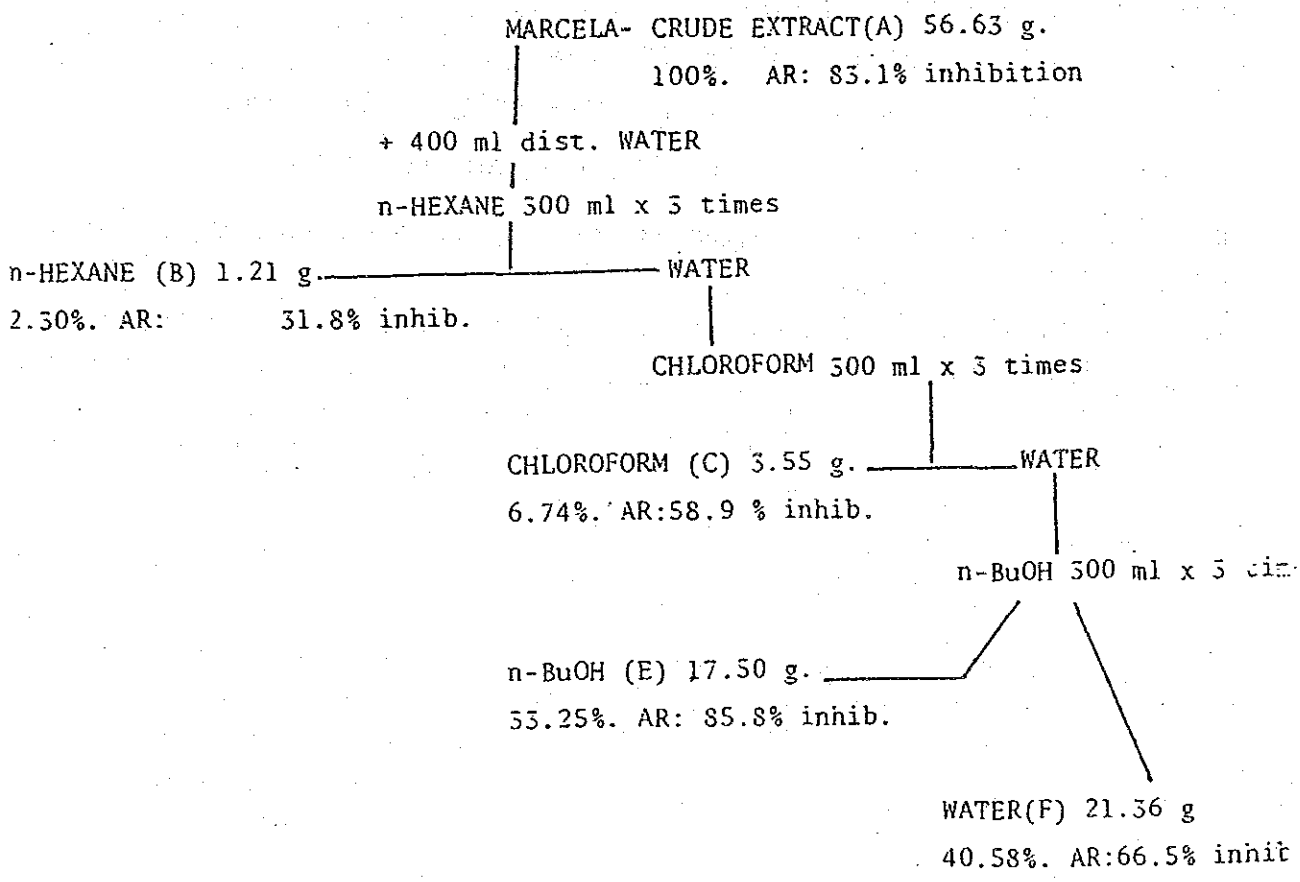
$$\text{Urease(Summer units/ml)} = \frac{50 \cdot f \cdot 0.4 \cdot n}{V \cdot t}$$

f: temperature factor
n: dilution % of the enzyme solution
V: volume of enzyme solution tested
t: time to change pH from 6.7 to 7.7

The urease is stable at 4° C. one month and 8 units/tube are used in each assay.The urease preparation was performed in the Hygienic Sciences Lab of this University.

SOLVENT FRACTIONATION OF A CRUDE EXTRACT OF MARCELA

52.63 g of an extract obtained from Marcela in room temperature conditions at the Faculty of Chemical Sciences(Paraguay) were suspended with 400 ml of water and sonicated during 20 min. The plant material was extracted using 70% aqueos Ethanol. The solvent fractionation was made at room temperature using n-Hexane,Chloroform and water saturated n-Buthanol(n-BuOH). Each fraction was washed with 50 ml. of water(100 ml of n-BuOH saturated water for the n-BuOH fraction)and these water washes were mixed with the water layer before the next solvent extraction. Each solvent was shaked 30 min(10 min x 3 times) before layers separation. The extraction scheme was as fallow , and for each fraction is showed the yield and the AR inhibition % at $1x10^{-5}$ g/ml.



MARCELA FRACTION	SOLVENT	AMOUNT(g)	YIELD(%)	AR INHIBITION (%)
A(crude extract)	70%EtOH	56.63	100	83.1
B	n-Hexane	1.21	2.30	31.8
C	$CHCl_3$	3.55	6.74	58.9
E	n-BuOH	17.50	33.25	85.8
F	H_2O	21.36	40.58	66.5

REFERENCES ABOUT THE GENUS CITHAREXYLUM (SARA MOROTI)

The search was made using the fallowing sources:

Konstitution und Vorkommen der Organischen Planzenstoffe. Walter Karrer Birkhauser Verlag(1958). Bassel.

Annual Index of the Reports on Plant Chemistry.
Hirokawa Publishing Company Inc. Tokyo.(1958-1972)

Chemical Abstracts.1970 untill now(July,1986- Vol 105, N° 1)

Was found one reference about Citharexylon genus.Also was searched the genus Verbenoxylon as a synoname.

" Citharexylum solanaceum,iridoids of,taxonomy in relation to" 91:189758w
Z.Naturforsch,C Biosci 1979,34C(5-6) 316-29.

LABORATORY REPORT. DEPARTMENT OF PHARMACOGNOSY. TOYAMA MADICAL AND PHARMACEUTICAL UNIVERSITY. TOYAMA.

BY: DR. ESTEBAN A. FERRO BERTOLOTTO
FROM: JULY,23,1986
UNTIL: AUGUST,22,1986.

During this month were performend the fallowing activities:
-Preparation of the AR enzyme from rat lenses(lot#5) and checking of the enzyme activity.
-Protein assay of AR preparations(lots #3,#4, and #5).
-Calculation of AR specific activity(lot #3).
-Fractionation of the n-BuOH fraction(E) of a crude extract obtained at room temperature from Marcela.
-Use of a Droplet Counter Current Chromatograph(DCCC).
-Assistance to the 6th Symposium on the Development and Application of Naturally Occurring Drug Materials(July,25-July,26) at Nagoya.

****

PREPARATION OF AR CRUDE ENZYME FROM RAT LENSES

From 26 Wistar rats (6-7 weeks old)were obtained the lenses, and the enzyme preparation was performed using the procedure reported previously. After the usual activity check and dilution, the crude enzyme was aliquoted in 3 fractions of 1 ml. and 5 fractions of 1.3 ml. and stored at - 25° C. This batch was labeled as Lot#5.

PROTEIN ASSAY OF ARPREPARATIONS(LOTS #3,#4 and #5)

The protein concentration of each lot was measured using the Bio-Rad Protein Assay Kit(Bio-Rad Laboratories,Richmond,USA),based on the diferential absortion of Coomasie Brillant Blue G-250 dye when it's bound to proteins.

The assay was performed using as protein standard bovine serum globulin(BSG**at different concentrations. The standard solutions and samples were diluted using a pH 7.2 buffer solution containing 6.8 g of KH_2PO_4 and 8.76 g of NaCl in 1000 ml of dist. water. pH was adjusted using KOH solution. Fallowing the assay procedure 0.1 ml of satndards and samples were placed in test tubes. The samples(AR crude enzyme) were diluted 1:50 using the pH 7.2 buffer solution. 0.1 ml of buffer solution was used as blank. 5.0 ml of the diluted dye reagent(1:5) were added to each tube and incubated at room temperature 30 minutes after gentle mixing. The absortins at 595 nm were measured using a Hitachi 220 Spectrophotometer, and the OD_{595} were plotted against standard concentrations to get a satandard curve for read the unknown values.For each standard concentration and sample were made 3 tubes and the values plotted are the average.

SAMPLE	PROTEIN CONCENTATION (MICRO G)	OD_{595}
Standard 1	153.0	0.989
Standard 2	107.1	1.009
Standard 3	76.5	0.741
Standard 4	45.9	0.493
Standard 5	22.9	0.269
AR Lot#3 (1:50)	79.0*	0.759
AR Lot#4 (1:50)	68.5*	0.668
AR Lot#5 (1:50)	59.1*	0.586

* From standard curve.

This assay gave for the AR lots the fallowing protein concentrations :

AR LOT#3: 3950 micro g = 3.95 mg/ml
AR LOT#4: 3425 micro g = 3.42 mg/ml
AR LOT#5: 2955 micro g = 2.95 mg/ml

**BSG = 1.53 mg/ml(stored at -78° C) . Stock solution.

AR SPECIFIC ACTIVITY (Lot#3)

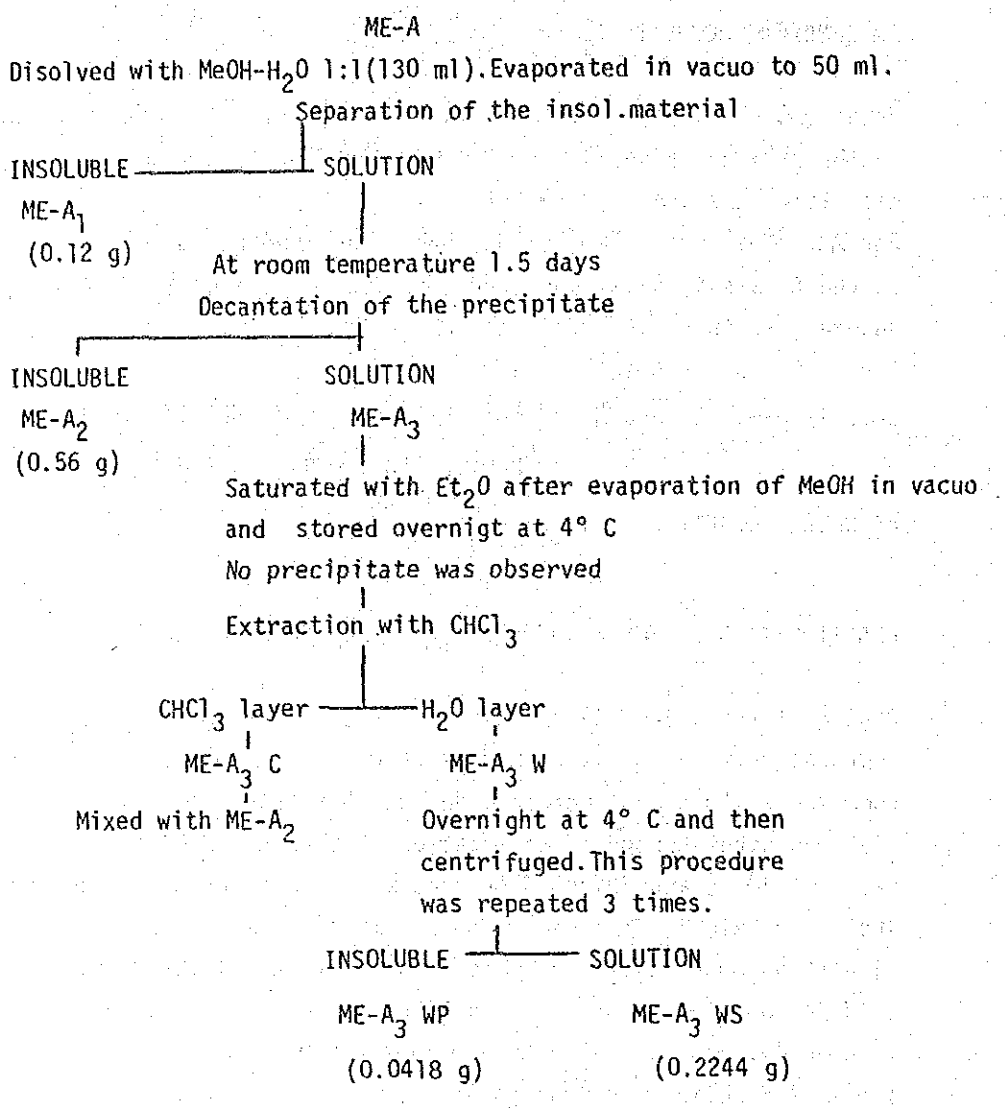
Using the previouly reported value for the AR(Lot#3) activity based on the rate of consuption of NADPH and the protein concentration,was calculated the specific AR activity.
For AR,1 Unit = 1 x 10^{-9} mol of NADPH consumed per minute.
In the AR assay conditions there is 1.45 Units (0.02 ml of AR crude enzyme), so there is 72.50 Units/ml.
For AR Lot#3, Protein = 3.95 mg/ml

$$\text{AR SPECIFIC ACTIVITY} = \frac{\text{AR ENZIMATIC ACTIVITY (UNITS/ML)}}{\text{AR PROTEIN CONCENTRATION (MG/ML)}}$$

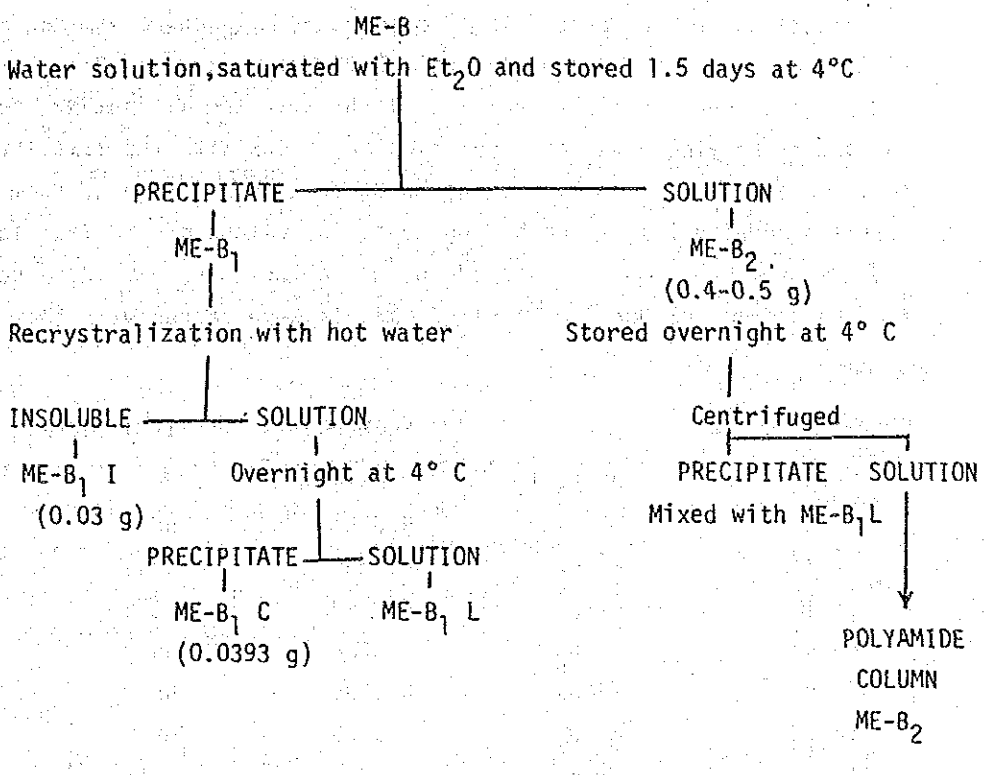
AR SPECIFIC ACTIVITY= 18.35 Units/mg

FRACTIONATION OF THE N-BUTHANOL (E) FRACTION OF MARCELA EXTRACT

2.06 g of the n-BuOH(E) fraction of Marcela extract,obtained at room temperature with 70% EtOH, were suspended in 30 ml of dist. water at room temperature and sonicated during 10 minutes. The insoluble material was separeted by centrifugation,disolved in MeOH,evoparated in vacuo at 40° C and dried. This fraction labeled ME-A weighs 1.43 g. The water soluble fraction wasmixed with 120 ml of MeOH,but no precipitate was observed.After evaporation in vacuo of the MeOH,was added cold Acetone to the water extract and no change was noted.The water soluble material ,labeled ME-B,weight was 0.60 g.
The ME-A fraction was suspended in 30ml of boiling dist. water, and after 10 minutes was filtered off . The hot water soluble material was mixed with ME-B for further separations and the insoluble material remains as ME-A. ME-A and ME-B fractions were treated separatelly according with the fallowing schemes.The fractions showing similar TLC patterns were mixed.



The TLC patterns were made using Silica Gel and Cellulose pre-coatted plates. Silica plates were eluted with the fallowing solvent mixtures EtOAc-MeOH 10:3 ; $CHCl_3$-MeOH-H_2O 35:65:40(lower layer) and $CHCl_3$-MeOH 2:1. The spots were observed under UV light,with and without NH_3 fumes and spraying 10% H_2SO_4 or AcOH-H_2SO_4-H_2O and heating at 100° C in both cases.



POLYAMIDE COLUMN ME-B_2

A clear water solution of ME-B_2 (about 0.5 g)was chromatographied in a column(2.5 cm Ø,35 cm long) filled with Polyamide C-200 (Wako Pure Chem.Ind) and packed with water.The column was eluted according with the fallowing scheme:

SOLVENT	AMOUNT (ML.)	FRACTIONS (ML.)
Water	500	--
MeOH-Water 10:90	600	1-2(300)
MeOH-Water 20:80	200	3 (200)
MeOH-Water 50:50	700	4-5(150) 6-7(50) 8-15(30)
MeOH-Water 70:30	600	16-24(30) 25-49(15)
MeOH	500	50-71(15) 72-78(50)
5% Na_2CO_3(water sol.)	300	80-92(15)

The fractions were checked using Ployamide TLC plates(Polyamide FM Plate,Wako Pure Chem.Ind) eluted with MeOH-Water mixtures(3:1, 5:1) and the spots were observed under UV light with and without NH_3 fumes and after spraying with $FeCl_3$ solution.Fractions with the same TLC pattern were mixed and labeled as ME-B_2 fractions . Some of them were tested with the AR inhibition test. The weight of the fractions and the AR inhibition % is showed in the fallowing scheme. Also some fractions from nBuOH(E) extract of Marcela were tested with that enzymatic assay.

COLUMN FRACTION	SAMPLE	WEIGHT(G)	AR INHIBITION %
7-18 19-20	ME-B_2 1+2	0.0628	NP
21-30	ME-B_2 3	0.0580	NP
31-34	ME-B_2 4	0.0209	67.9
35-48	ME-B_2 5	0.0692	58.6
49-53	ME-B_2 6	0.0082	55.8
54-57	ME-B_2 7	0.0095	NP
58-66	ME-B_2 8	0.0159	NP
67-70	ME-B_2 9	0.0056	64.3
71-73	ME-$B_2$10	0.0108	NP
74-77	ME-$B_2$11	0.0163	67.8
78-79	ME-$B_2$12	0.0061	57.6
80-83	ME-$B_2$13	0.1215	83.5
84-92	ME-$B_2$14	0.0643	NP
-----	ME-A_3 WP	0.0418	76.8
-----	ME-A_3 WS	0.2244	85.2
-----	ME-B_1 C	0.0393	61.9

The AR inhibition test was performed using the usual procedure,and the samples were tested in DMSO solution of 10 micro g/ml. A standard sample of Quercitrin was tested twice at 1 micro g/ml giving a AR inhibition % of 67.8.- The AR enzyme belongs to the Lot #3.

** NP= not performed.

COMPARISON OF SARA MOROTI CRUDE EXTRACTS

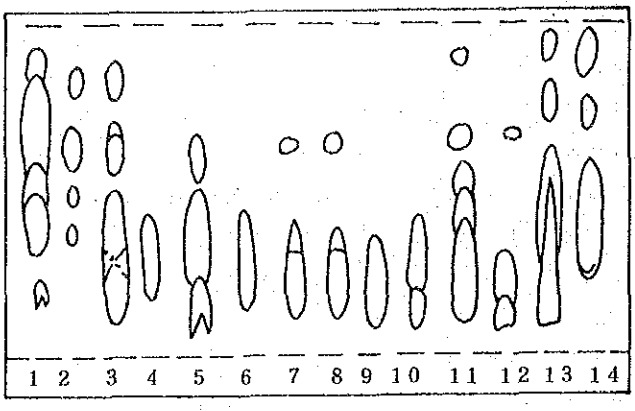
Two different crude extracts from bark of Sara Moroti,obtained both in hot extraction conditions were tested with the AR inhibition test as showed in the first report. Since the inhibition % value of the samples was different , TLC of both was performed using Silica Gel plates eluted with $CHCl_3$-MeOH 3:1 and observed under UV ligth and after spraying with AcOH-H_2SO_4-Water 80:10:10 and heating. Also TLC with cellulose plates eluted with n-BuOH-AcOH-Water 4:1:2 and observed under UV light(w. & wo. NH_3 fumes)and after spraying with $FeCl_3$ solution was made. Both extracts show the same main spots,but the first one collected at the National University Campus(SanLorenzo) exibit a bigger amount of chlorophil and low polarity compouds. The second sample collected at Capiataseems to be from an older plant. Compairson of these extracts with the samples purchased from the market should be done using chemical and biological methods.

USE OF D.C.C.C.

A short trainig was made using the droplet counter current chromatograph. The solvent system was $CHCl_3$:MeOH:Water 35:65:40. The lower layer was used as stationary phase and the upper layer was used as mobile phase. A sample containing a dye mixture of Guinean Green, Naphtol Yellow and Ponceau-SX was separated.(1.7 mg of sample in a mixture 1:1 of both layers).The equipment was set for working with 120 tubes, and 10 ml fractions were collected with a fraction collector working overnight. All the operations for the previous set up of the apparatus were performed.

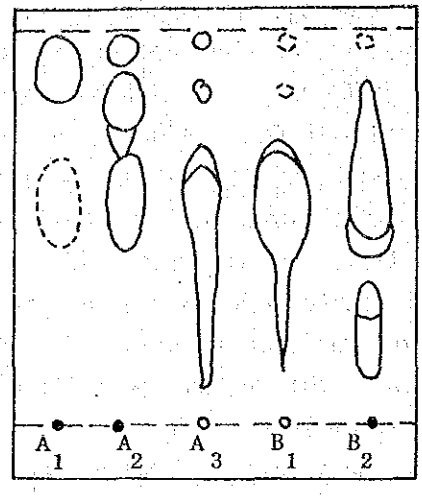
SUMMARY OF SOME TLC PLAFES

MARCELA(E)
POLYAMIDE COLUMN FRACTIONS(ME-B_2)

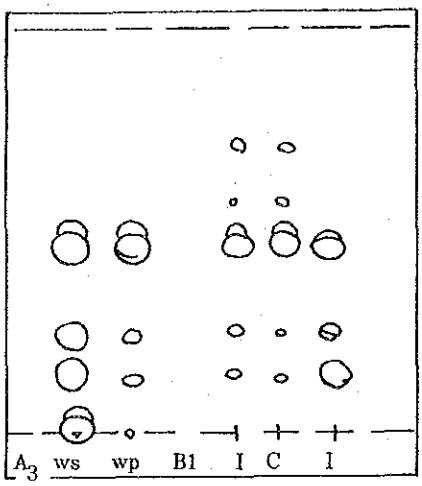


Polyamide FM. MeOH-H_2O 5:1. UV light

MARCELA(E). WATER SOL/ INSOL. FRACTIONS

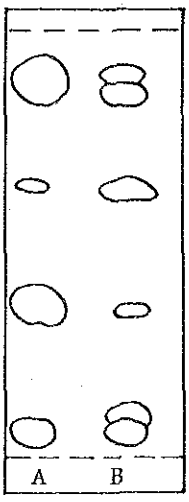


Cellulose. $CHCl_3$-MeOH-H_2O
65: 25:4, uv light



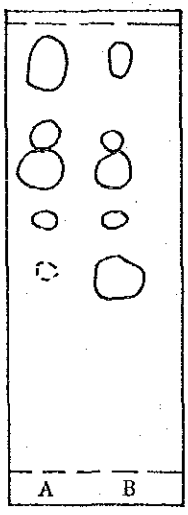
MARCELA(E)
Silica Gel
$CHCl_3$-MeOH-H_2O 35:65:40(Lower layer)
UV light

SARA MOROTI CRUDE EXTRACTS

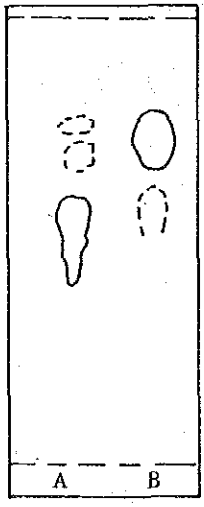


Silica Gel
$CHCl_3$-MeOH
3 : 1
UV light

(1)



(2)



Cellulose
n-BuOH-AcOH-H_2O
4 : 1 : 2
(1): UV light
(2): $FeCl_3$

A=Campus sample
B=Capiata sample

phytochemistry から C/P Miss Lucia Franco 報告書
1985年7/26～10/3 まで研修

Final report about the training course in Toyama (Japan)

Place: Toyama Medical and Pharmaceutical University - Japan

Field: Phytochemistry

Chief of the department: Prof. Dr. Noakata Morita

Instructor: Prof. Dr. Munehisa Arisawa

Duration of the training course: From July 26th to October 3rd (1985)

Part one

- Extraction - purification and structural elucidation of active principles focussed on flavonoids
- Cromatography methods
- Determination of melting point
- Spectroscopic methods: Basic training on Ultraviolet Spectra (U.V.)
  Infrared Spectra (IR) Nuclear Magnetic Resonance (N.M.R.)
- Hydrolysis methods (Acidic Hidrolysis)
- Acetilation methods

Finally was presented a report concerning to this part of the training course.

Part two

Bioassays (in vitro)

Test 1: Inhibition of Angiotensin Converting Enzyme (ACE)

- Procedures for determining the protein concentration of the enzyme solution.
- Determination of the enzyme concentration required for the assay.
- Determination of the incubation time optimum.
- Determination of the inhibition of Angiotensin Converting enzyme by Captopril.

  Calculation of IC50

I have never tried this assay using a plant extract.

Test 2: Cytotoxicity against KB cells

- Methodology of cells culture: Medium used . Counting cells method.
- Assay of cytotoxicity against KB cells:

  Determination of inhibition percentage. ED50

  Material: a) Simaba multiflora

  b) Acanthospermum australe

Visits to other institutions

8 - Aug - 85

- Visit to Togamura (observation of growing plants in high places).

30 - Aug - 85

- Visit to Kokando Pharmaceutical Company.

9 - Sep - 85

- Symposium of Medical and Pharmaceutical Society (Wakun-Yaku) in Kyoto City.
- Visit to Kyoto Botanical Garden.

Angiotensin converting enzyme (ACE)

Assay is based on:

Hippuryl ⟨ L histidyl-L-Leucine ⟶ Hippurate + L-histidyl-L-Leucine

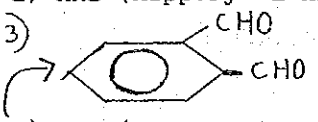
ACE

L-histidyl-L-Leucine: quantified spectrofluorimetrically

Hippurate + L-histidyl-L-Leucine: Fluorescent adduct with o-phtbaldialdehyde

1) Angiotensin convertin enzyme
   Rabbit lung acetone power
   (by Sigma Chemical Co.) (1g.)

2) HHL (Hippuryl-L-histidyl-L-Leucine)
   (by Sigma Chemical Co.) (1g.)

3) 

3) OPA (*o-phtaldialdehyde*) for biochemistry
   (by Nakarai Chemical Co.)

4) 0,3 N NaOH (S.G.)

5) 3 N HCl (S.G.)

6) Buffer solution

Type A
1) KH_2PO_4 (S.G) 0,34 g/50 ml. H_2O
2) K_3PO_4 (S.G) 0,53 g/50 ml. H_2O

Instead of K_3PO_4, use $K_3PO_4 \cdot 3H_2O$: 0,665 g/50 ml. H_2O

Type B
1) KH_2PO_4 2,04 g, NaCl 3,50 g | 100 ml. H_2O

2) K_3PO_4 3,18 g
* ($K_3PO_4 \cdot 3H_2O$ 3,99 g)
NaCl 3,50 g / 100 ml. H_2O

Type C 1) KH_2PO_4 6,80 g
NaCl 8,77 g
/ 500 ml. H_2O

2) K_3PO_4 10,61 g
* (K_3PO_4. 3 H_2O 13,31 g)
NaCl 8,77 g
/ 500 ml. H_2O

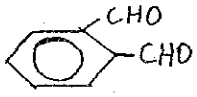
All type 1) + 2) ⟶ PH 8,3

Reagents

1) 3N HCl C. HCl 128,75 ml/H_2O 500 ml.
d= 1,18

2) 0,3 N NaOH NaOH 6g/ H_2O 500 ml.

3) OPA 2,0 W/V %

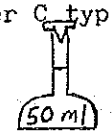


20 mg / MeOH 1ml.
(on occasion)

4) Substrate (HHL) (Mw = 429,47)

Hippuryl-Histidyl-Leucine

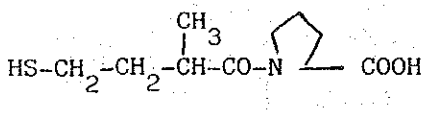
107,375 mg/ Buffer C type 50 ml.
* Dissolve in



5) Enzyme Powder 1 g/ Buffer A type 20 ml.

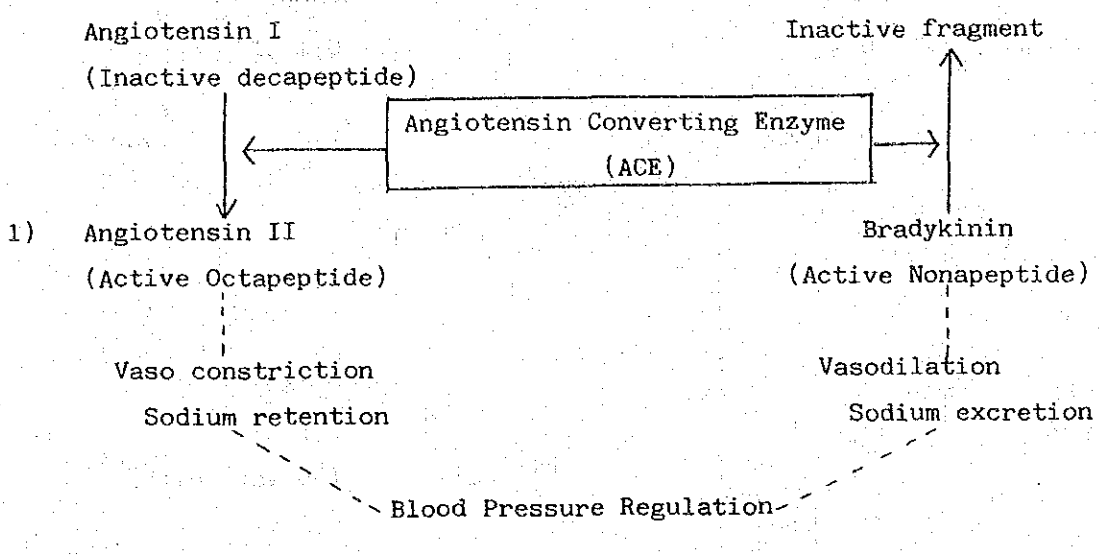
Homogenized and then ultracentrifugated

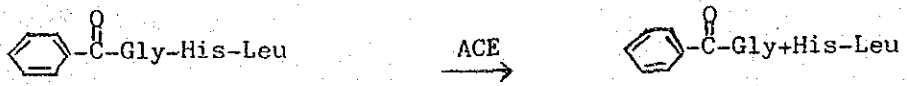
6) Captopril



MW 217

Reactions catalyzed by ACE



2) 

Protein assay

Standard Assay Procedure

Prepare several dilutions of protein standard (Standard: 1,32 mg/ml), and enzyme solution.

1. Place 0,1 ml of standards in test tubes and 0,1 ml of buffer type C in blank test tube.
2. Add. 5,0 ml diluted dye reagent (Ej: 20 ml + 80 ml H_2O)
3. Vortex
4. Incubation. After a period of 30 minutes (37°C) measure OD 595.
5. Plot OD 595 vs concentration of standards. Read un Knowns from the standard curve.

Enzyme concentration assay

Enzyme concentration prepared: 1/5, 1/10, 1/15

Blank Buffer type B

Procedure:

	Enzyme		Blank	
Substrate (HHL)	0,1 ml		0,1 ml	
Enzyme	0,14 ml		---	(vortex)
		Incubation 37°C 30 min		
NaOH 0,3 N	1,45 ml		1,45 ml	(vortex)
? Enzyme	—		0,14	
2% OPA	0,1 ml		0,1 ml	
		10 min	(no wat. bath)	
HCl 3N	0,2 ml		0,2 ml	
	read			

Plot Intens vs concentration of enzyme solution and choose the best concentration.

Incubation time

15, 30, 45, 60 minutes

Procedure:

	Enzyme		Blank
Substrate	0,1 ml		0,1 ml
Enzyme (1/15)	0,14 ml		---
		Incubation 37°C	
NaOH 0,3 N	1,45 ml		1,45 ml
Enzyme	---		0,14 ml
2% OPA	o,1		0,1
		10 min	
HCl	0,2		0,2
	340 nm	455 nm	

Ploted Intens vs time and choosed the best incubation time

Inhibition of ACE by captopril

Captopril doses (with Buffer C) Ex:

$C_1 = 1{,}28 \cdot 10^{-7}$ mol/l

$C_2 = 6{,}4 \cdot 10^{-8}$ mol/l

$C_3 = 2{,}56 \cdot 10^{-8}$ mol/l

$C_4 = 1{,}28 \cdot 10^{-9}$ mol/l

Enzyme solution: 1/15

Procedure

	Enzyme (E)	blank (B)	Sample	Sample blank
Substrate	0,1	0,1	0,1	0,1
Sample (Captopril solution)	---	---	0,01	0,01
Buffer C	0,01	0,01	---	---
Enzyme	0,14	---	0,14	---
		↓ Incubation 30 min.		
NaOH 0,3 N	1,45	1,45	1,45	1,45
Enzyme	---	0,14	---	0,14
OPA 2%	0,1 ml	0,1 ml	0,1	0,1
		↓ 10 min.		
HCl 3 N	0,2	0,2	0,2	0,2

Calculation

$$\text{Inhibition (\%)} = \frac{E - S}{E} \times 100$$

E = with out inhibitor

S = sample with inhibitor (captopril)

IC 50 = $1{,}8 \cdot 10^{-7}$ mol/l

Reference: A sensitive fluorimetric assay for Serum

Angiotensin. converting enzyme

Joan Friedland PH D et al

Am. J. Clin. Pathol, 66.416 (1976)

ACE - Assay method

Sample: 2,5 mg/1ml DMSO $\longrightarrow$ 100 µg/ml

His-Leu Mw=252 # Assay method :

	A Control	B Control Blank	C Sample	D Sample blank
Substrate	0,1	0,1	0,1	0,1
Sample	---	---	0,01	0,01
DMSO	0,01	0,01	---	---
Enzyme	0,14	---	0,14	---
	$37^{\circ}C$ 30 min incubation			
0,3 N NaOH	1,45	1,45	1,45	1,45
Enzyme	---	0,14	---	0,14

OPA 0,1 ml

↓

10 min

↓

3N-HCl 0,2 ml

↓

Measure { Emission fluorescence wavelength:455 nm

Excitation wavelength : 340 nm

$$\text{Inhibition (\%)} = \frac{(A - B) - (C - D)}{A - B} \times 100$$

Note: If we get very high inhibition we can change the concentration of sample with another one; but if we get very small inhibition with this concentration, we musn't change the first concentration

C/P Dr. D. Ibarrola 研修報告

(1986. 5/10 ~ 1987. 5/9)

Ref: Pharmacological Training at Department of Pharmacology, Faculty of Medicine, Toyama Medical and Parmaceutical University
(From May to December in 1986)

A) Experiments of Biochemical Pharmacology

Hepatocytes from male rats of Wistar strain were isolated by separate perfusion with collagenase by the method of Nakanishi et al.. Kinetic studies on aldehyde dehydrogenase, alcohol dehydrogenase, glutamate dehydrogenase, cytochrome c oxidase, NADPH-cytochrome c reductase and glucose-6- phosphatase were carried out according to the method of Lineweaver and Burk, using mitochondrial, microsomal and cytosolic fractions of isolated hepatocytes.

B) Experiments of Physiological Pharmacology

I) Intact Preparation

Anaesthetized dogs were used. The femoral artery,femoral vein,and trachea were cannulated and ureter was catheterized. Mesurements of blood pressure,respiration activity,heart rate and urine volume,were carried out after administration of drugs.

II) Isolated Preparations

The frog heart (Yagi-Hurtung's method) and the intestine of rabbit and guinea-pig (Magunus's method) were used. In both methods the responses to the drugs were recorded mechanically. The drugs have been added to the organ bath.

Conclusion

I have considered that the training at the department of pharmacology is very efficient to success the purpose of the JAPANESE- PARAGUAYAN project. Because all these experiments mentioned above could be applied to the pharmacological studies of medicinal plants.

XI　結　　語

薬草の化学薬学的研究が1985年5月よりスタートし、日本から各技術専門家が現地の国立アスンシオン大学赴き、言語、環境、また研究機材や、水、電気などの不備を克服して、両国相携えて協力研究を行ない、植物学では市場品薬草の起源植物名が同定出来たこと、植物化学においては、薬草の薬効による生物実験（酵素実験）で各種の作用を見出すことの出来たこと、そして現地大学で初めてスタートする薬理学の基礎を当大学で有意義に研修されるなど、この1年半における成果はみるべきものがあったと信ずる。

あと残り少ない期間は三部門の研究推進はもとより、パラグアイ薬草の標本園薬草園を作り上げなければならないこと、そしてまた、パラグアイ薬草のカラー写真集を立派に製本し、研究の実績と供に残すことはきわめて意義あることである。

この研究に当たり、機材供与その他、大変な御援助いただいた国際協力事業団、および現地、国立アスンシオン大学化学部のスタッフに対し、心から感謝の意を表する次第である。