3.2.6　C／Pに対する技術指導

現地で植物の研究の一環として、染色体観察方法について指導を鈴木専門家が行った。表－20の実験マニュアルを作成し、時間的関係でその中で基本的なAceto-carmine染色法について試薬の調製、おしつぶし、顕微鏡観察方法を指導した。他の染色方法についても基本的には同一であり、植物の種類ごとの最適条件の確立など試行錯誤による他はないと考える。

その他写真撮影、現像、腊葉標本の作製、生薬標本の作製、見本園植物の名札の件などを各専門家が適宜指導を行った。

表－20　染色体観察法、実験マニュアル

How to make 2% Aceto-Orcein (2% Aceto-Carmine) staining solution

1) Boil 99% acetic acid (22.5 ml) and cool to 95°C
2) Add Orcein (1.0 g) or Carmine (1.0 g)
3) Boil gently for 30 minutes and cool to 50°C
4) Add distilled water (27.5 ml)
5) Filtrate
6) Store (cool and dark)

How to make Schiff's staining solution

1) Distilled water (80°C, 100 ml)
2) Add Basic Fuchsin (1.0 g)
3) Boil gently for 10 minutes and cool to 50°C
4) Filtrate and cool to 25°C
5) Add Sodium Metabisulfite (2.0 g)
6) Add 1N-HCl (20 ml)
7) Store (cool and dark)

Aceto-Orcein (Aceto-Carmine) Method

These solutions can stain cytoplasm, nucleus and chromosome

1) Clean the material (root tip 5-10 mm) with water
2) To get many metaphase chromosomes, soak the material in 0.002·M 8-Hydroxyquinoline for 2-6 hours
3) Soak the material in the fixing solution for 1-24 hours.

   (Fixing solution)

   a) 45% Acetic acid solution
   b) Ethyl alcohol:Acetic acid= 3:1
   c) Ethyl alcohol:Acetic acid:Chloroform= 6:3:1
   d) 10% Formalin

4) To soften the material, soak in 1N-HCl (60°C) or 1N-HCl(2) +45% Acetic acid(1)(60°C) for 10-30 seconds
5) Place a drop of Aceto-Orcein solution (or Aceto-Carmine solution) on a clean slide glass, and add the softened material
6) With a pair of iron needles tease the material apart and place a cover slip over the preparation
7) Apply pressure to the cover slip to flatten the material
8) The preparation is now ready for examination

Feulgen Method

Schiff's staining solution can stain DNA

1) Clean the material (root tip 5-10 mm) with water

2) To ge many metaphase chromosomes, soak the material in 0.002 M 8-Hydroxyquinoline for 2-6 hours

3) Soak the material in the fixing solution for 1-24 hours

(Fixing solution)

a) 45% Acetic acid solution
b) Ethyl alcohol:Acetic acid = 3:1
c) Ethyl alcohol:Acetic acid:Chloroform = 6:3:1
d) 10% Formalin

4) To soften the material, soak in 1N-HCl (60°C) or 1N-HCl(2) +45% Acetic acid(1)(60°C) for 10-30 seconds

5) Rinse with water

i)	70% Ethyl alcohol	5-10 minutes	room temparature
ii)	30% Ethyl alcohol	5-10 minutes	room temparature
iii)	15% Ethyl alcohol	5-10 minutes	room temparature
iv)	Distilled water	5-10 minutes	room temparature
v)	Distilled water	5-10 minutes	room temparature

6) Macerate with HCl

i)	1N-HCl	5 minutes	room temparature
ii)	1N-HCl	8-10 minutes	60°C
iii)	1N-HCl	5 minutes	room temparature
iv)	Distilled water	5 minutes	room temparature
v)	Distilled water	5 minutes	room temparature
vi)	Distilled water	5 minutes	room temparature
vii)	Distilled water	5 minutes	room temparature

7) Staining with Schiff's staining solution

i)	Schiff's staining solution	1-2 hours	room temparature
ii)	10% Sodium Metabisulfite*	5 minutes	room temparature
iii)	10% Sodium Metabisulfite	5 minutes	room temparature
iv)	10% Sodium Metabisulfite	10 minutes	room temparature

8) Place one or two drops of 45% Acetic acid solution on a clean slide glass, and add the stained material

9) With a pair of iron needles tease the material apart and place a cover slip over the preparation

10) Apply pressure to the cover slip to flatten the material

11) The preparation is now ready for examination

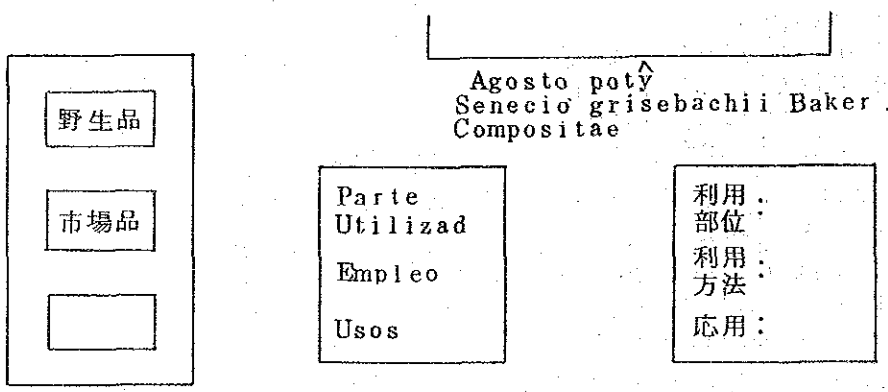
*:10% Sodium Metabisulfite = 10% Sodium Metabisulfite solution (5 ml)+1N-HCl(5 ml)+Distilled water(100 ml)

3.2.7　薬草写真集の作製 ……プロジェクトの一環として

「パラグァイの薬草」写真集の発行準備し、現在までに市場品、野生品あわせて約1200枚のスライド（複数の同一種を含む）を撮影した。しかし、地理的条件により野生品の比重は小さく、市場品（アスンシオン第4市場）を中心とした写真集になる。すでに、予行集を刊行したが、新たに250種程度の「パラグァイ薬草」写真集を刊行する予定である。C／Pとも協議し 表－21に示した案にそったものとする。

表－21　最終的な『パラグァイ薬草の写真集』案

- まえがき（プロジェクトの説明、スタッフ、利用方法、応用の記載は聞き取り、etc）
- パラグァイ薬草の利用状況（植物の科別利用種、適用症状別の利用植物種、テレレ・マテ茶の説明、テレレ・マテ茶と薬草利用との関係）
- 写真（スタッフ、アスンシオン大学の風景、施設）
- 写真（市場風景、テレレ・マテ茶の飲用風景・器具、薬草の採取・栽培）
- 写真（市場品、野生品）→現地名によるアルファベット順



- アンケート結果（アンケートの説明、結果、まとめ）
- あとがき
- 索引（学名、スペイン語、日本語）

※　スペイン語、日本語の対訳形式とする。

3.2.8　研究材料の収集調製

このプロジェクトの薬草研究協力で、初年度は種々の点で誤解があったがC／Pとのミーテングを重ね解決した。一方、生薬学的研究の方法について話し合った。お互に連携がとれ植物学からの材料供給、研究材料の取り扱い方がお互に理解できた。

4. 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.

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

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).

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

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 250-260 minutes after disolution of NADPH. So the useful time for assay is arround 3 hours.

For the calculation of inhibition % was used the formula:

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

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 %
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 (?)
Guayaba $5x10^{-6}$	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

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.

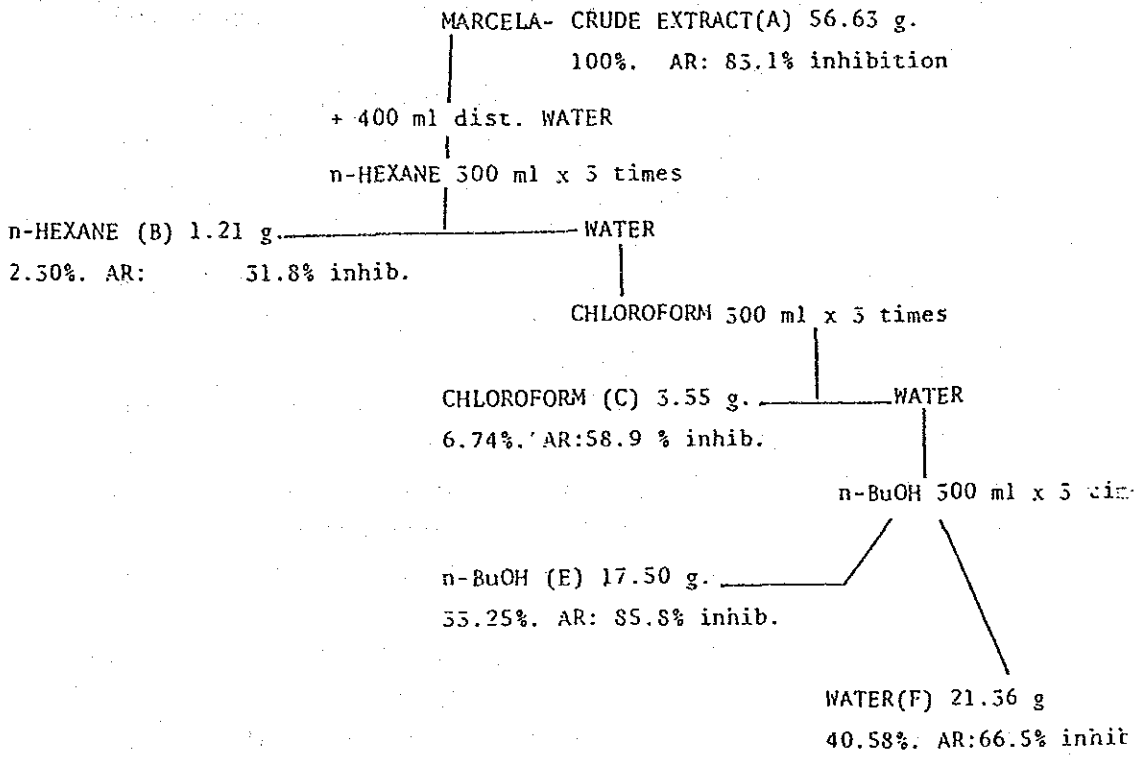
ε: $\varepsilon_{NADPH} = 6.22 \times 10^{6}\ cm^{2}/mol$

b: path length = 1 cm

c: concentration of NADPH.

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.



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.

AR SPECIFIC ACTIVITY (Lot#3)

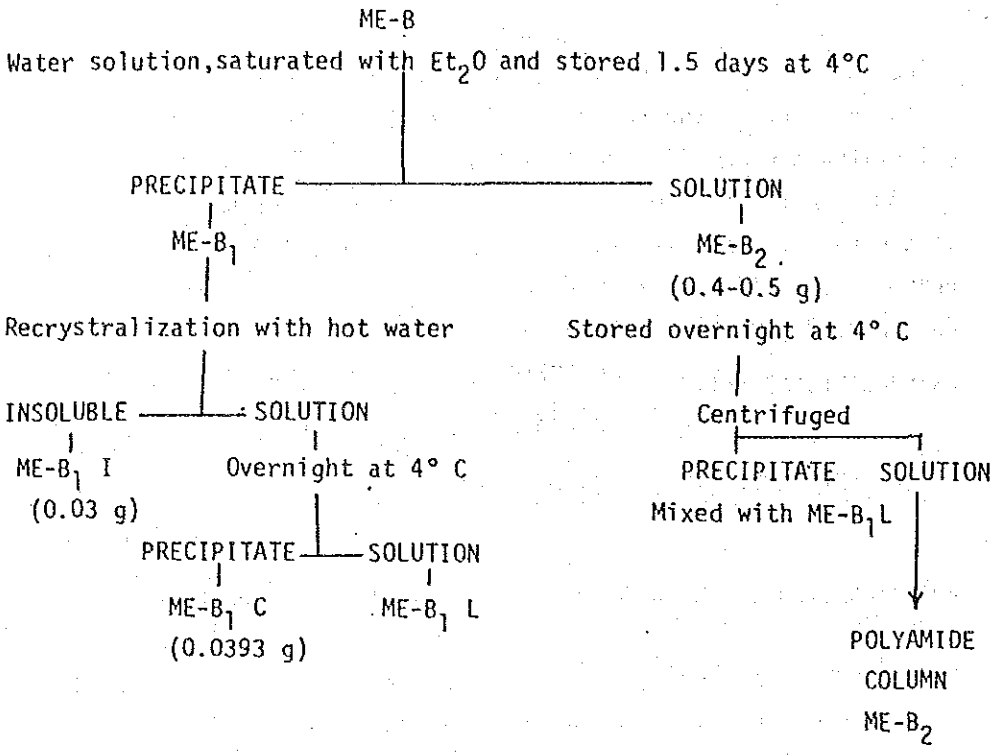
Using the previouly reported value for the AR(Lot#3) activity based on the rate of consuption of NADPH and the protein concentration,was calculated the specific AR activity.
For AR,1 Unit = 1 x 10^{-9} mol of NADPH consumed per minute.
In the AR assay conditions there is 1.45 Units (0.02 ml of AR crude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



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)

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.

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: 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)

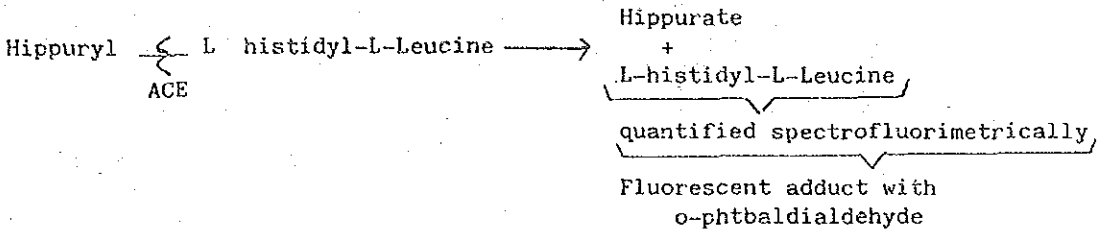
- Procedures for determining the protein concentration of the enzyme solution.
- Determination of the enzyme concentration required for the assay.
- Determination of the incubation time optimum.
- Determination of the inhibition of Angiotensin Converting enzyme by Captopril.

Calculation of IC50

I have never tried this assay using a plant extract.

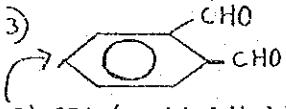
Angiotensin converting enzyme (ACE)

Assay is based on:



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) 

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

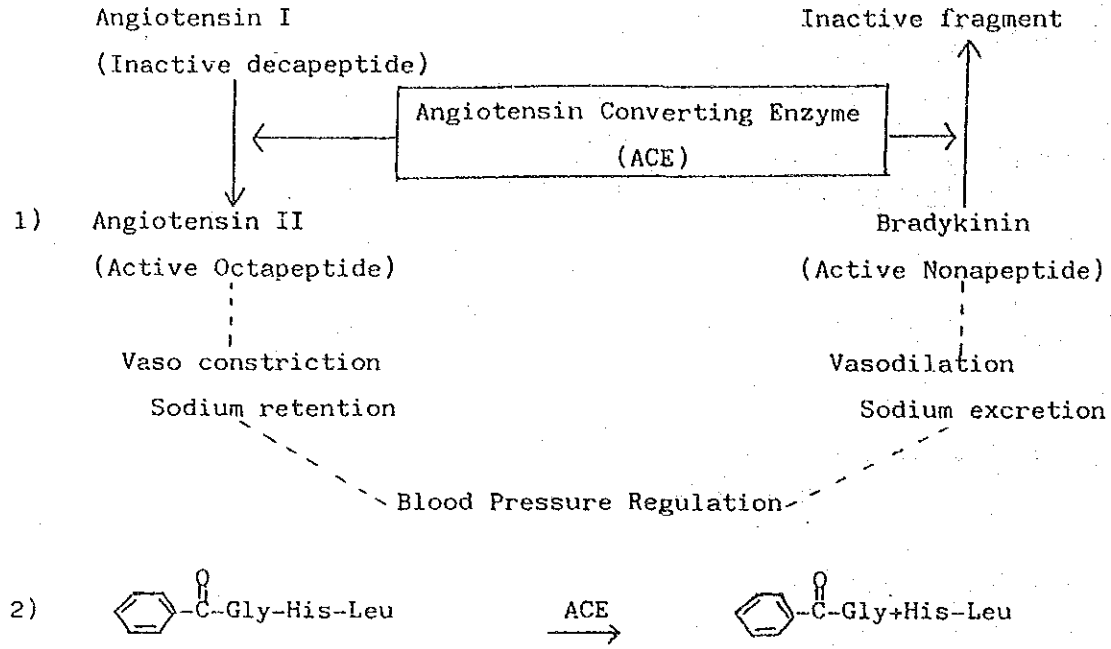
Type A
1) KH_2PO_4 (S.G) 0,34 g/50 ml. H_2O
2) K_3PO_4 (S.G) 0,53 g/50 ml. H_2O

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

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

Inhibition of ACE by captopril

Captopril doses (with Buffer C) Ex:

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

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

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

$C_4 = 1{,}28 . 10^{-9}$ 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 = $1{,}8 . 10^{-7}$ mol/l

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.

Ⅳ ま と め

1. パラグァイ国への協力効果について

1.1 具体的研究成果

i) 具体的研究　パラグアイの伝承薬草を市場から、また野生品を含め、10ケ月にわたり280種の薬草を採集した。これら薬用植物を腊葉標本に作製管理した。次に採集した280種について、現地人から聞きとり調査を行って、薬効の整理をした。（前述）、供与機材（顕微鏡、電気泳動装置、コンピューター）の整備により、薬草の組織学的、育種学的研究が行われるところとなった。以上から薬草の植物学的分類が行れることゝなり、従来分類されてきたパベッティ博士の標本、パラグアイ植物園、アメリカ、アリゾナ州立植物園、ブラジル、アルゼンチン各大学などの標本と比較、同定確認を行った。しかし未同定のものについては、さらに検討中である。

研究2年目の後半から各種の薬草を1個所に集中する薬草園の造成は着々と進捗し、薬草や薬木など植付け、また水生植物は池を作ってとり入れ、圃場には散水のスプリンクラーの設備も完備し、給水に万全を期し、研究最終の評価で昭和63年4月17日に現地に立派に完成したことは大きな喜びであった。薬草園に関し、今後の目標は、特定のよく使用される薬草（たとえば、Typycha Kuratu）を一定の土地で一定の条件のもと、肥培管理して、品質を一定に保ち、大量に栽培を進め、パラグアイ国の医薬品として出荷することは、薬の産業の活性化のため、きわめて大事なことである。

ii) 化学薬学的研究

薬草の伝承経験的な薬効をもとにして、しかも日常よく用いられている薬草の中で、高血圧症、肝臓病、糖尿病、リウマチ、腎臓結石などに関与する酵素阻害作用からみた生物基礎実験と癌細胞の増殖抑制をみるKB cell やL 5178 Y cell などを用いた方法も行ない、また抗炎症作用物質の研究など各種にわたった研究で、前述の如く大きな成果をあげ学会に報告することが出来た。本研究で10有余報の論文をあげることが出来た。

1.2 本研究の知名度および浸透について

パラグアイ薬草の化学・薬学的研究は本学薬学部薬用資源講座、薬用植物園、および医学部薬理学講座の教官、大学院生、学生、研究生らが総力を挙げて研究を遂行し、学会や地方例会など併せて15回にわたり研究の報告を行った。薬学会北陸支部例会（昭和62年11月14日）で本研究リーダー（森田）として特別講演を行い、本研究を報告することが出来た。本研究の論文は14報学会発表8報に達し、Chem、Pharm、Bull，薬学雑誌、生薬学雑誌、J，Natur、Prod：Plant、Medica，Phyto-chemistry などに論文を掲

載した。近時薬学会発行誌ファルマシア11月号（昭和63年11月１日）にパラグアイ研究のグラビア写真３頁や薬事日報（昭和63年７月21日号）で紹介されるなどこの研究に関する浸透度は高いものがある。また研究知名度も高い。

2. 研究選定の適正について

2.1　選定の理由

元パラグァイ総領事杉田敏次氏は富山市の出身で、自らの糖尿病がパラグァイ薬草で癒ったこと、その他各種の疾病に有効な薬草が多数存在することを知り、そしてまたこれら薬草の化学的研究がおくれていることもあり、これが開発のため、本学へ国際協力事業団を通じ研究協力依頼をうけ、応じたものである。

2.2　研究の規模、期間、などについて

本研究は小規模プロジェクトである。薬草の化学薬学的研究は少くとも５～10年であれば、まとまった研究は可能と思われるが、本研究は３ケ年と限定され、とくに最初の１年目は現地大学の研究室の場を作るのに費し、市場で売られる薬草や野生のものなどの蒐集に10ケ月要し、標本の作製、整理に時間を要し、化学的研究する材料を大量に採集出来る場所を見出すことや、採集日数を要し、乾燥に日を要し、初年度は不慣れな土地の環境、習慣などに慣れることもあって、まずは研究する土台作りに終始したわけである。この土台、基礎を元にして、実際の研究は２年目、３年目と行い、研究は進展し、本学研究室では３ケ年にわたり、教官４名、大学院生数名、学部４年生18名たちの大きな協力によって遂行することが出来た。植物学研究は薬草園では教官２名、技官３名、学生数名で、また薬理学関係では教官４名などで行われ、すべて研究室挙げて研究遂行し成果をあげた。

3. 進行管理について

3. 1　専門家派遣およびC／P（研修員）受入れ

次表のごとく３ケ年の計画書

Schedule

Item ＼ year	phase 1 1985.5～1986.4	phase 2 1986.5～1987.4	phase 3 1987.5～1988.4
(1) Team Leader, Pharmacognosy Dr., Prof, Morita	0.1M 4/11 4/21 0.5M 4/10 4/25		4/6 4/19
(2) Pharmaceutical Botany Dr., Aso. Prof, Yoshizaki	10M 7/1 4/25		2/28 3/31
(3) Phytochemistry Dr., Aso-Prof., Shimizu	2M 5/10 7/10	0.5M 10/27 11/16	
(4) Phytochemistry Dr. Arisawa	2M 5/10 7/10	0.5M 7/20 8/9	
(5) Phytochemistry Dr. Hayashi	9/10 11/9	6M 4/10 10/14	
(6) Plant Breeding Dr. Suzuki	1M 5/10 6/8	2M 1/27 3/31	1.5M 7/24 9/8
Mr. Yoshiaki Tatuo		2M 1/27 3/31	
Mr. Hiroharu Fujino			7/24 9/8
Mr. Norihito Yamazaki			2/28 3/21
(7) Pharmacology Dr., Prof., Nakanishi			0.5M 4/6 4/19
Dr., Momose			1M 11/10 12/10
Miss, Yamazaki			1M 11/30 12/30
C/P from Paraguay			
(1) Botany, Dr. Isabel Basualdo		3M 5/10 8/2	
Miss Nelida Soria	2M 7/10 9/10		
Miss Milta Ortiz	2M 7/10 9/10		
(2) Phytochemistry Dr. Esteban Ferro		6/22 8/22	
Miss Lucia Franko	2M 7/10 9/10		
(3) Pharmacology Dr. Ibarrola		14M 5/10 7/15	
Dr. Lucia Areco			2M 5/15 7/15

3.2　機材および現地業務費について

3ヶ年の研究において、植物学、植物化学、薬理学など三分野において現地で研究出来る最低の研究機材の適正配置が出来た。また化学実験、動物実験で現地で調達出来ない薬品類や酵素などについては国際協力事業団の方から充分に手配を終った。そして現地で調達出来る薬品、試薬、その他消耗品などについては、現地業務費で充分手配出来た。（表9）

3.3　現地の受入体制について

化学研究室で抽出中の薬草不足の場合、植物学研究室に採集を依頼しても容易に協力をしてくれない。化学の方ですることはそちらでという意識が強いようで連携のまずさが眼についた。現地は発展途上国であり、大学の教官は午前中研究すれば午後はいないとか、また逆の場合もあり、このようなことは、大学での低月給のために出稼ぐという国情と判明した。日本からの専門家は朝早くから夜おそくまで研究に精を出していることと比較すると、全く問題にならない情況である。この様な情況に合わせなければならないし、我慢し努力したわけである。このような姿は亜熱帯や熱帯における発展途上国の一つの姿であるのかもしれない。

3.4　本邦での支援体制について

現地大学からのC／P（研修員）受入れにあたっては、本学の教室に入る研修費は少く、むしろ教室費から出る経費の方が多いのではと思われる。そんな点を考慮いたさき、昭和62年度、（研究3年目）本学前学長佐々学先生から、このわれわれプロジェクトに対し、学長決裁による200万円の援助うく、大きな理解である。

4. 相手国の対応について

4.1　C／P（研修員）について

三ケ年の間で現地大学植物学研究室の方から、Dra.I.Basualdo：S.Nelida：O.Miltaの3名が2～3ケ月、植物化学の方からDr.E.Ferro：L.Francoの2名が2ケ月、薬理学研究室からは、この部門は現地大学化学部で始めての開講で、先ず基礎の把握のためにDr.D.Iballoraが1年3ケ月間、本学医学部薬理学教室で研修をうけ、またL.Arecoさんがついで来日、2ケ月間薬理実験を研修した。この三つの部門の中、薬理学が一番おくれており、これが充実は大で、本研究終了後も、さらに教育指導が大切で、研修の大幅継続をすべきである。

4.2　大学の実施体制

現地大学の教官の給与水準は低く、アルバイトで生計の補助をする人が多く、したがって1日を通しての研究時間は少く、研究論文を作るという、本来の研究態勢にないということが日本と大きなちがいである。したがって本研究は相互の研究協力であるが、研究材料およびそれらの抽出エキスを作る操作の協力をいたゞき、本学が研究を推進して論文を作り上げ、論文著者らの中に共同研究の意で、現地大学の部門の長の名を連らね協調に万全を期した。

9. 供与機材

s.	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 PROJECTOR (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-15131P)	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 (FE-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, 12.5/100 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 2.5㎜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 № 2 225 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 Buketa 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 pc	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 8㎜ × 1,200㎜	5 〃	180	900
126	Glass Tube 8φ × 1,200㎜	15 〃	100	1,500
127	—do— 10φ × 1,200㎜	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㎖ 3,500 R.P.M. (32pcs)			
	*—ditto— 50㎖ 4,000 R.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 (500㎖)	6 〃	¥ 550	3,300
150	METHYL ALCOHOL (500㎖)	18 〃	¥ 400	7,200
151	HYDROCHLORIC ACID (500㎖)	5 〃	¥ 470	2,330
152	ACETIC ACID (500㎖)	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 (500㎖)	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 300㎖	10 pcs	¥ 400	4,000
169	-ditto- 500㎖	10 〃	¥ 580	5,300
170	-ditto- 1 L	5 〃	¥ 1,080	5,400
171	-ditto- 2 L	3 〃	¥ 2,200	6,500
172	-ditto- 3 L	2 〃	¥ 2,900	5,300
173	STIRRING PICKUP ROD TEFLON	1 pc		2,650
174	REAGENT BOTTLE 250㎖	5 pcs	¥ 1,500	7,500
175	-DITTO- 500㎖	5 〃	1,900	9,500
176	-DITTO- 1 L	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 30cm × 10m	3 pcs	@¥ 680	2,040
230	CAPILLARY TUBE FOR DISPENSER	1 pc		3,500
231	ALUMINIUM FOIL 30cm × 25cm	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 × 17m/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

5. むすび

5.1 技術の移転について

国際協力事業団の発展途上国に対する技術援助は、それぞれのプロジェクトの内容に応じて行れるもので、それら研究に必要とする機材が供与され、技術専門家を派遣することによって、技術を移転し援助することである。本薬草の研究は3ケ年にわたり行われ、植物学、植物化学、薬理学の三つの部門において研究を進めてきた。その中、薬理学は始めての開講と、しかも研究2年目からのスタートで完全に出おくれてしまった。これが専門家の育成は1年半の育成指標のみで、これでは不足であり、このあとまだ継続すべきで、3年間は必要である。

5.2 計画の遅延

本研究の実施計画からみて、薬草の植物学的な分類、鑑定においては、約90%の植物を鑑定した。未鑑定の植物については、国内、国外の権威者に協力方をお願いし、早急に分類を解決する予定である。

化学薬学的研究では、現地で繁多の薬草約60種の抽出エキスを作り、これを酵素を用いた生物実験を行い、阻害作用を有する有効成分を抽出分離し、その化学構造の決定を行った。

薬理学の研究は前述の如く現地大学化学部でスタートしたばかりで専門家がいないため、本研究では2年目（昭和61年度）からC／P（研修員）を本学医学部薬理学講座へ派遣させ、1年3ケ月間にわたり研修を行い、基礎薬理学の一部を学んだにすぎない。このようなことであり、前述の化学研究で有効成分の化学構造を決定したが、これら成分を動物実験をすべき観点において、薬理的研究のおくれは遺憾という外はない。今後さらに研修を進め対応出来ることが切にのぞまれる。

5.3 研究終了後の予測

パラグァイ薬草の化学薬学的研究は3ケ年にわたり、日本とパラグァイ国両国大学の協力共同研究が行われた。現地パラグァイの伝承薬草の薬効について生物実験を（各種病態に関係の酵素阻害の研究）行って有効成分を確認し、それらの化学構造を決定出来たことは大きな成果であった。

薬理学は現地大学化学部で始めての開講で、本学医学部で1年3ケ月の研修をうけ、基礎的薬理学を学んだにすぎない。植物化学の研究で薬草の有効成分が判明し、次いで動物実験によって薬効の確立をはかる必要があるのに、薬理学研究の不備は残念である。したがってこの専門家の一日も早い養成はきわめて大切である。現在1人（男性）の研修を1年

3ヶ月行い、1人（女性）は2ヶ月行って帰国したが、もう1人（男性）の若い研究家の養成が大事で、現地大学で人員の確保が要望される。

5.4 写真集の出版

3ヶ年に行った薬草の写真集（200種）を出版出来たことは大きな成果であった。

おわりに

パラグァイ国薬草の化学薬学的研究はJICA支援の下、昭和60年4月～昭和63年3月までの3ケ年間、パラグァイ国立アスンシオン大学化学部研究科と当富山医科薬科大学薬学部薬用資源学講座、医学部薬理学講座および薬用植物園などとプロジェクトを作り、現地の市場で売られている各種の薬草を蒐集し、標本作りを行い、その分類研究で280種の薬草を整理した。それら薬草の化学的研究は、平素よく用いられている薬草60種について、抽出エキスを作り、これを用いて各種病気に関連ある酵素の阻害作用をみる生物実験を遂行した。すなわち、肝機能、糖尿病関連の白内障に関係、高血圧、腎結石、リウマチなど関係の酵素阻害作用、癌細胞の増殖抑制をKB、L－5178 Y cell を用いて調べる方法などの他、抗炎症作用（抗ヒスタミン作用も）などなどについても研究を遂行し、阻害作用の強い薬草のエキスを化学的に有効成分を分離、分割し有効成分を明らかにして、それらの化学構造を決定することが出来た。

また有用薬草を一個所に集め栽培し、啓蒙するため、15000 m^2の薬用植物園を完成し得たことは本研究での大きな成果と信ずる。そして次に有用植物を一定条件下で肥培管理することによって、品質の高い薬草を生産することが出来、この生薬はパラグァイ国にとって、大きな薬産業の発展になるであろう。

まだまだ、未知の多くの薬草の宝庫である。本研究は氷山の一角の仕事をしたのみであり、今後、もっともっと研究の要あることを痛感した。

本研究は当大学プロジェクトの専門家はもとより、教室員全員一丸の成果であり、熱心な努力に対し感謝の意を表する。3ケ年間にわたる本研究で、日本とパラグァイ国の間に太い肝を結ぶことが出来たと信ずる。最後に大きな御支援を下さった外務省、国際協力事業団に対し深甚な謝意を表します。

学 会 報 告

① 日本薬学会第 106 年会　1986. 4　千葉

② 日本薬学会第 107 年会　1987. 4　京都

③ 日本薬学会第 108 年会　1988. 4　広島

④ 日本薬学会第 34 回年会　1987. 10　大阪（吹田）

⑤ 日本生薬学会第 33 回年会　1986. 10　埼玉

⑥ 日本薬学会北陸支部第 73 回例会

⑦ 日本薬学会第 107 年会　1987. 4　京都

⑧ 第 29 回天然有機化合物討論会　1987. 8　札幌

⑨ 日本生薬学会第 34 回年会　1987. 10　大阪（吹田）

⑩ 日米合同薬学大会　1987. 12　ホノルル

⑪ 日本薬学会第 108 年会　1988. 4　広島

⑫ 日本生薬学会第 33 回年会　1986. 10　埼玉

日本薬学会第106年会　1986.4　千葉

＜学会報告1＞

2D 9-5　パラグァイ薬草の化学薬学的研究　第1報
TAPECUE'(Acanthospermum australe)のAldose Reductase阻害活性成分

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吉崎正雄、川崎　勝、寺島　恵、辻　秀樹、和田修治
植野　一、森田直賢
アスンシオン大　L.H. Berganza, E. Ferro, I. Basualdo

〔目的〕　パラグァイには伝統的な自然治療法があり、そこで使われる伝来の興味ある薬草が数多く知られている。今回入手し得た21種の薬草について、各種生物活性試験を行った結果、外用として、はれもの等に有効であるといわれているTAPECUE'(Acanthospermum australe)に、カラゲニン浮腫抑制作用及びβ-qlucuronidase阻害活性が認められたほか、特に強いaldose reductase(AR)阻害活性が認められたので、その活性成分について検討を加えた。

〔方法〕　AR阻害活性(in vitro)の検定は既報に従った。[1)]

〔結果〕　'TAPECUE'の70%アルコール抽出エキスを水に懸濁し、n-ヘキサン、クロロホルム、n-ブタノールで順次分配したところ、n-ブタノール画分に活性が移行(IC_{50}：1.5×10^{-6} g/ml)したので、ポリアミドカラムクロマトグラフィー及びトヨパールHW40Fカラムクロマトグラフィーを用いて分画を行った結果、6種の化合物を単離した。そのうち3種は、各種スペクトルデータより、4',5,7-tri hydroxy-3,6-dimethoxy flavone (1)、hyperin (2) 及びquercetin (3) と推定し、標品との直接比較により同定した。何れも本植物より初めて単離されたものである。今回得られた6種の化合物について、AR阻害活性を調べたところ、1に最も強い活性(IC_{50}：10×10^{-7} M)が認められた。また2及び3についても活性が認められた。

1) M Shimizu et al. : Phytochemistry-23 (9), 1885-1888 (1984)

日本薬学会第107年会　1987.4　京都

＜学会報告2＞

2Ag　1−2　パラグァイ薬草の化学薬学的研究　第5報

Aldose Reductase 阻害活性物質

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鈴木正一、吉崎正雄、川崎　勝、植野　一、和田修治
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アスンシオン大　L. H. Berganza, E. Ferro, I. Basualdo

〔目的〕　パラグァイ薬草の化学薬学的研究の一環として、これまでに入学した約60種のパラグァイ薬草について数種の活性スクリーニングを実施したが、その中の15種に比較的強いaldose reductase（AR）阻害活性作用が見出された。今回その中のPara-paraimí（Phyllanthus riroo L.）、Saráモロティ（Citrarexybun myriarthum cran.）、及びMarcela（Acryooooo sacueioides（Lam.）D.C.）の活性成分について報告する。

〔実験・結果〕　Para-paraimi（全草）、Saraモロティ（葉）、及びMarela（地上部）を70％EtOHで抽出し、Chart 1のように分画した。Para-paraimiでは沈殿部（B）が最も強い活性を示し、カラムクロマトグラフィーによりellagic acid（1）（IC_{50}；2.0×10^{-7}M）他2種の化合物を活性物質として単離した。一方、Saráモロティの最も強い活性を示したη-BuOH画分（E）から4,5,6−trihydroxy−3,7−dimethoxyflavone及びその6−qlucosideを、Marcelaの活性画分（E）からluteolin, quercetin, isoquercitrin, quercimeritrin、他3種のフラボノイドを活性物質としてそれぞれ単離した。

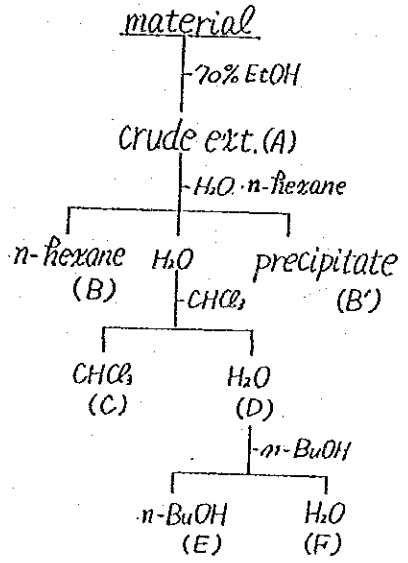


Chart 1.

上記3種の薬草のうちSaráモロティ，Para-paraimíは糖尿病の治療を目的としても利用されており、AR阻害活性がみられたことは興味深い。

日本薬学会第108年会　1988.4　広島

＜学会報告3＞

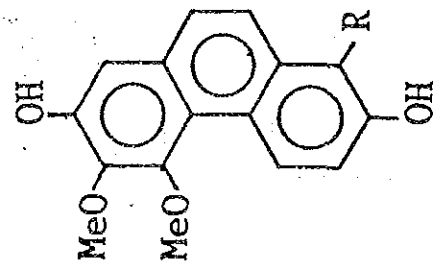
6E$_{12}$　10-1　パラグァイ薬草の化学薬学的研究　第10報

Tamarndá cuná の消炎活性成分

富山医薬大・薬　清水岑夫、○正川　仁、林　利光、有澤宗久、鈴木正一
吉崎正雄、森田直賢
アスンシオン大　L. H. Berganza

〔目的〕　パラグァイ薬草の化学薬学的研究の一環として局所適用による抗炎症作用が認められたTamandá cuná (Catasetum barbatum Lindle, Ochidaceae) の70% EtOH熱時抽出エキスについて活性成分の検討を行った。

〔実験・結果〕　パラグァイで腰痛、神経痛、気管支炎等の治療に利用されているTamandá cuná 地上部の70% EtOHエキスを常法により液性分画し、局所適用によるカラゲニン足浮腫試験を行ったところ、弱酸性画分に活性が移行した。また、モルモット摘出回腸収縮抑制試験においても弱酸性画分にのみ活性が集中したので、微量で行えるこのassay法を指標として4種の化合物（1～4）を単離した。1は無色粉末でmp 158℃ UV, ^{1}H-NMR 等の所見より、2,7－dihydroxy－3,4－dimethoxyphenan threneと結論した。2は無色粉末でmp 197-199℃ UV及び1とそのdiacetateの^{1}H-NMRとの比較より、2,7－dihydroxy－3,4,8－trimethoxyphenanthreneと推定した。本化合物の天然からの単離報告は初めてである。3及び4は各種スペクトルデータよりそれぞれ2,7－dihydroxy－3,4－dimethoxydihydrophenanthrene、5,4′－dihydroxy－3,3′－dimethoxydihydrostilbeneと推定した。4種の化合物中1及び2に比較的強い活性が認められたので類似phenanthrene誘導体の合成並びにそれらの活性についても検討中である。



1：R=H
2：R=OMe

日本生薬学会第34回年会　1987.10　吹田
＜学会報告4＞

15-I-B-17

パラグアイ薬草の化学薬学的研究　第6報
Alhucema(Lavandula latifolia Vill.)の消炎活性成分

富山医薬大・薬　清水孝夫，○正川仁，松沢孝泰，林利光，有澤宗久，川崎勝，鈴木正一，
吉崎正雄，森田直賢　アスンシオン大　L.H.Berganza, E.Ferro, I.Basaldo

【目的】パラグアイ薬草の化学薬学的研究の一環として，これまでに入手した数十種のパラグアイ薬草について一次スクリーニング（抗炎症ならびに数種の酵素阻害試験）を行ない，そのうち数種に抗炎症作用が見いだされた。今回，現地において気管支炎等の治療目的に利用され，その70%EtOH抽出エキスに局所適用によるカラゲニン浮腫抑制効果が認められたAlhucema（Lavandula latifolia Vill.）の活性成分の検討を行った。

【実験方法・結果】Alhucema全草の70%EtOH抽出エキスを水に懸濁し，$CHCl_3$で抽出したところ$CHCl_3$可溶部にのみ活性が移行し，沈殿部および水可溶部には全く浮腫抑制効果を認めなかった。そこで，$CHCl_3$可溶部を$CHCl_3$-MeOH系を展開溶媒としたシリカゲルカラムに付し，1%MeOH溶出部（fr.1），3%MeOH溶出部（fr.2），10%MeOH溶出部（fr.3），50%MeOH溶出部（fr.4）に分画し，それぞれの活性試験を行なったところursolic acidを大量に含有するfr.2及びfr.3，fr.4には抑制効果はなく，fr.1にのみ集中した。fr.1から活性成分を単離すべく，遠心クロマト，シリカゲルカラム及びPreparative TLCを繰り返し，fr.1の5分の1に相当するcoumarin，及び微量成分としてこれまでにtrans-phytol，caryophyllene oxide，7-methoxycoumarinをそれぞれ単離した。

量的に得られたcoumarin及びfr.1について活性試験を行ったところ（Table 1），coumarinには弱い浮腫抑制効果がみられたものの，fr.1の活性を説明できるものではなく，現在微量成分の量産をはかるとともに他成分についても検討中である。また，ヒスタミンによるモルモット摘出回腸収縮抑制試験においてもfr.1に弱いながら活性があり，単離した化合物の中でcaryophyllene oxideが$IC_{50}=4.2\times10^{-5}$Mを示した。

Table 1　Inhibitory Effect on Carrageenin Edema in Rat

Treatment[a)]	Dose (mg/site×4)	Inhibition of Swelling Parcent (%)			
		1hr[b)]	2hr	3hr	4hr
70%EtOH ext.	5	0.0	18.1	30.1*	18.5
$CHCl_3$ ext.	5	31.8	10.0	32.5**	24.3*
fr.1	3	44.8**	47.0**	45.5**	50.1**
coumarin	5	20.5	11.5	22.6*	19.0
indomethacin	0.5	5.1	15.8	37.3*	33.8*

a) Topically applied to the hind paw
b) Time after carrageenin injection
Significantly different from control, *$p<0.05$, **$p<0.01$

日本生薬学会第33回年会　1986.10　埼玉

＜学会報告5＞

1A　4－5　パラグアイ薬草の化学薬学的研究　第2報
Romeroのウレアーゼ阻害およびKB細胞増殖抑制について

(富医薬大・薬)○林利光，有澤宗久，長留恵司，南百瀬佳也，清水岑夫，鈴木正一，吉崎正雄，川崎勝，植野一，堀江俊治，和田修治，五川仁，藤田尊大，寺島恵，森田直賢，(アスンシオン大)L.N. Berganza, E. Ferro, I. Basualdo.

〈目的〉　パラグアイ薬草の化学薬学的研究の一環として，主に労者性健胃，利尿を目的に使用されているromero (*Rosmarinus officinalis* L., Labiatae) の生物活性成分の検索を行ったので報告する．

〈実験方法・結果〉　Romeroの地上部を乾燥・粉末化し，70%EtOHで抽出後chart 1. のように分画した．Urease阻害作用及びKB cell増殖抑制作用を示したn-hexane可溶部(A)と沈殿部(B)についてカラムクロマトグラフィーによる成分分画を行った．その結果，構造既知のrosmanol, carnosol, genkwanin, ulsolic acid及びbetulic acidとともに新物質として黒色針状晶(1)，mp 218－223℃，$C_{32}H_{50}O_5$ が単離された．化合物(1)は各種スペクトルデータより7-ethoxyrosmanolであると推定した．単離した成分についてurease阻害試験及びKB cell増殖抑制試験を実施したところ，rosmanolに弱いurease阻害作用が認められ，7-ethoxyrosmanol及びbetulic acidに弱いKB cell増殖抑制作用が認められた．

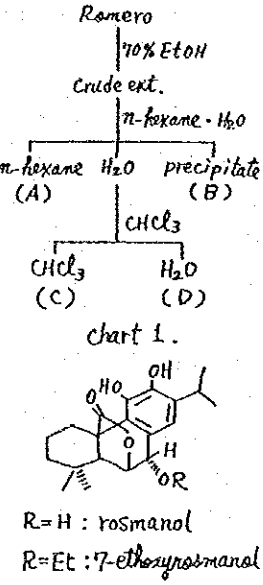


Chart 1.

R=H : rosmanol
R=Et : 7-ethoxyrosmanol

日本薬学会北陸支部第73回例会

＜学会報告6＞

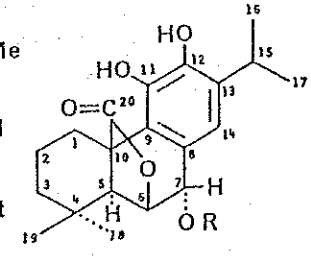
パラグアイ薬草の化学薬学的研究　第8報
Romero（Rosmarinus officinaris L.）について　（その2）

富山医薬大・薬　有澤宗久、林　利光、○大村和伸、長山勝良、
清水岑夫、森田直賢、アスンシオン大・化　L. N. Berganza

【目的】パラグアイ薬草の化学薬学的研究の一環としてRomero（Rosmarinus officinalis L., Labiatae）の生物活性成分の検索を行っているが，その過程でcirsimaritin，betulinの他に新化合物を単離したので，それらについて報告する。

【実験・結果】Romero地上部の乾燥，粉末化した材料を70%EtOHで抽出し，得られたエキスをn-hexane－H2Oで分配して，n-hexane可溶部，H2O可溶部及び沈殿部に分画した。H2O可溶部を更にCHCl3で抽出して得られたエキスをシリカゲルカラムクロマトで分離し，3種の化合物（1－3）を単離した。1は黄色針状晶，mp 264－268℃，で，フラボン反応は陽性であり，そのUV，PMRなどからcirsimaritinが予想されたので，標品と直接比較し同定した。2は無色針状晶，mp 248－250℃，〔α〕+11.63°，で，Liebermann－Burchard反応は陽性を示し，PMR，MSなどからbetulinが予想されたので，文献記載のスペクトルデーターとの比較から同定した。3は淡褐色粉末状，〔α〕-99.2°，で，そのFeCl3反応は陽性を示し，UV，IRなどはrosmanol(4)や7-ethoxyrosmanol(5)のそれらに類似している。分子式はhigh MSよりC21H28O5と決定された。PMRは4や5のそれらに類似しているが，δ 3.66ppmにOMe 1個分のシグナルがみとめられる点が4と異なる。また，CMRにおいてもδ 58.2ppmにOMeに基づくシグナルが存在する。これらの知見や4，5のCMRとの比較から7-methoxyrosmanol(3)と決定した。

3；R＝Me
4；R＝H
5；R＝Et



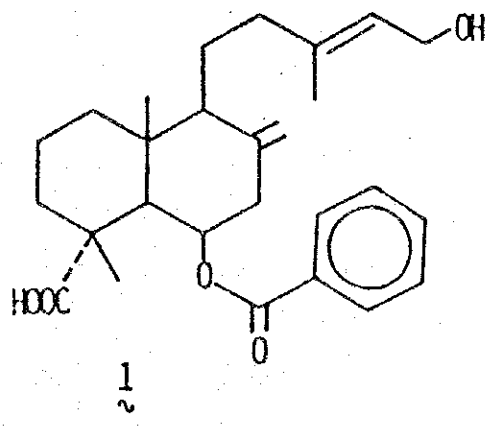
日本薬学会第107年会　1987.4　京都
＜学会報告7＞

3Ah 9-1　パラグアイ薬草の化学薬学的研究　第4報
Typychâ Kuratü (scoparia dulcis)の活性成分の検索

富山医薬大・薬　○川崎　博，林　利光，有澤　恭久，清水　孝夫，堀江　俊治，植野　一，
和田　雄治，正川　仁，南百瀬　佳也，鈴木　正一，吉崎　正雄，森田　直賢，
岡・和漢研　手塚　康弘，鎌池　徹，Asuncion大・化　L.N.Bergarza，E.Ferro，I.Basaldo

［目的］　パラグアイ産薬草の抽出エキスの生物活性試験に於て，β-glucuronidase(β-glu)及びaldose ruductase(AR)阻害作用，KB細胞増殖抑制作用，及び抗炎症作用を示したTypychâ Kuratü (Scoparia dulcis L. Scrophulariaceae)の成分研究を行った。

［実験方法・結果］　エキスの分画・成分の分離精製方法はβ-glu阻害作用を指標にして行った。Typychâ Kuratü の全草の70%EtOH抽出エキスを水に懸濁し，n-hexaneで分画する際に生じた沈澱をシリカゲルカラムで分画した(Fr.I～IX)。最も強い活性を示したFr.IXに就いて更にシリカゲルカラムを繰り返し，最終的にはHPLCで化合物(1)を単離した。化合物1の2D-INADEQUATE，^{1}H-^{1}H COSY，^{13}C-^{1}H COSY，Longrange ^{13}C-^{1}H COSY，NOE等のNMRスペクトルの解析及び各種誘導体のスペクトルとの比較等により化合物(1)の構造を図の様に推定した。本化合物にはβ-glu，AR，Xantine oxydase阻害作用は認められず，弱いながらKB細胞増殖抑制作用及び抗ヒスタミン作用が認められた。すでに本植物より単離報告されているFriedelin，glutinol，α-amyrinにもβ-glu阻害作用は認められなかった。



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第29回天然有機化合物討論会　1987.4　札幌

＜学会報告8＞

72　パラグァイ産生薬 Typychá kuratũ(Scoparia dulcis L.)に含まれる新規ジテルペノイドの構造

富山医薬大・薬　○林　利光、　川崎　勝、　岸三重子
有沢宗久、　清水孝夫、　森田直賢
同・和漢薬研　手塚康弘、　菊池　徹
アスンシオン大・化　Luis H. Berganza

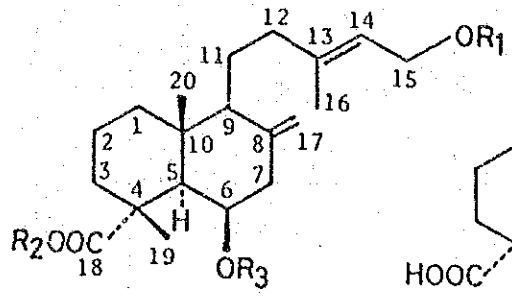
南米のグァラニーインディオの伝統薬物は現在でもパラグァイを中心に繁用されている。演者らはパラグァイ薬用植物に関して日パ両国の研究協力を行っているが、今回、生物活性スクリーニングにおいてβ-glucuronidase および aldose reductase 阻害作用、細胞毒性、抗炎症作用等が認められたTypychá kuratũ（ゴマノハグサ科、Scoparia dulcis L.）について成分研究を行った。Typychá kuratũ（全草）はパラグァイでは消化、健胃、肝機能改善薬として利用されている。なお、本植物は台湾やインドにも分布し、それぞれ高血圧および糖尿病等に用いられている[1,2]。今回、本植物より単離した新規ジテルペンカルボン酸 Scoparic acid A (1)、B (2)、C (3)、Scopadulcic acid A (4) および B (5) の構造について報告する。

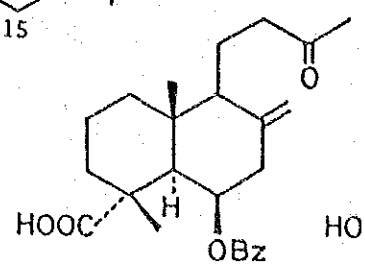
1．ジテルペンカルボン酸類の単離

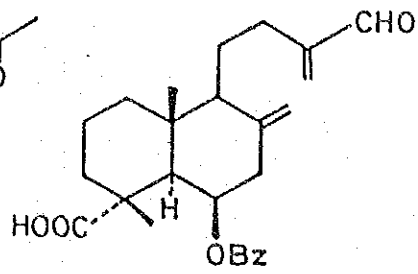
乾燥したTypychá kuratũの 70% EtOH 抽出エキスを水とn-ヘキサンで分配した際に生じた沈殿部の $CHCl_3$ 可溶部についてシリカゲルカラムクロマトグラフィーや PLC を繰り返し行い、本植物より既に単離、報告されている friedelin、glutinol、α-amyrin、betulinic acid[3,4] とともにジテルペンカルボン酸 1 - 5 を単離した。

2．ラブダン型ジテルペンカルボン酸類の構造

a) Scoparic acid A (1)、無色粉末、$[\alpha]_D$ -38.3°($CHCl_3$)、$C_{27}H_{36}O_5$ (HRMS, M⁺+1: 441.2670) はIR吸収より水酸基 (3500 cm^{-1})、カルボニル基 (1700 cm^{-1})、および二重結合 (1600, 1580 cm^{-1}) の存在が推定され、UVにおける 227、265、270、277 nm の極大吸収と MSにおける m/z 122、105、77のフラグメントピークの存在より、ベンゾイル基をもつことが予想された。^1H-および ^{13}C-NMR[5] よりビニルメチル基、ヒドロキシメチル基、エキソメチレン基、三置換二重結合、フェニル基（各 1 個）および三級メチル基、カルボニル基（各 2 個）の存在が示唆された。1 は CH_2N_2 で処理







1: $R_1 = R_2 = H$, $R_3 = COC_6H_5$
7: $R_1 = H$, $R_2 = CH_3$, $R_3 = COC_6H_5$
8: $R_1 = Ac$, $R_2 = H$, $R_3 = COC_6H_5$
9: $R_1 = R_2 = R_3 = H$

2

3

$Bz = COC_6H_5$

するとメチルエステル (7) を与え、Ac_2O-Py で処理するとモノアセテート (8) を与えた。また、1 は通常の方法では加水分解されず、封管中 KOH-MeOH-DMSO で加水分解することによりはじめて脱ベンゾイル体 (9) が得られた。以上の実験データと ^1H-^1H、^1H-^{13}C COSY の解析から 1 は部分構造 A および B をもつジテルペンカルボン酸であることが示唆された。これらの部分構造および他の炭素間の連結様式は 1 の 2-D INADEQUATE および ^1H-^{13}C long-range COSY の解析により明らかとなった(Fig. 1 および 2)。Scoparic acid A の相対配置は NOE差スペクトルおよび結合定数より 1 式のように推定した[6]。

b) Scoparic acid B (2)、無色粉末、$C_{25}H_{32}O_5$、$[\alpha]_D$ -9.8° ($CHCl_3$)、IR

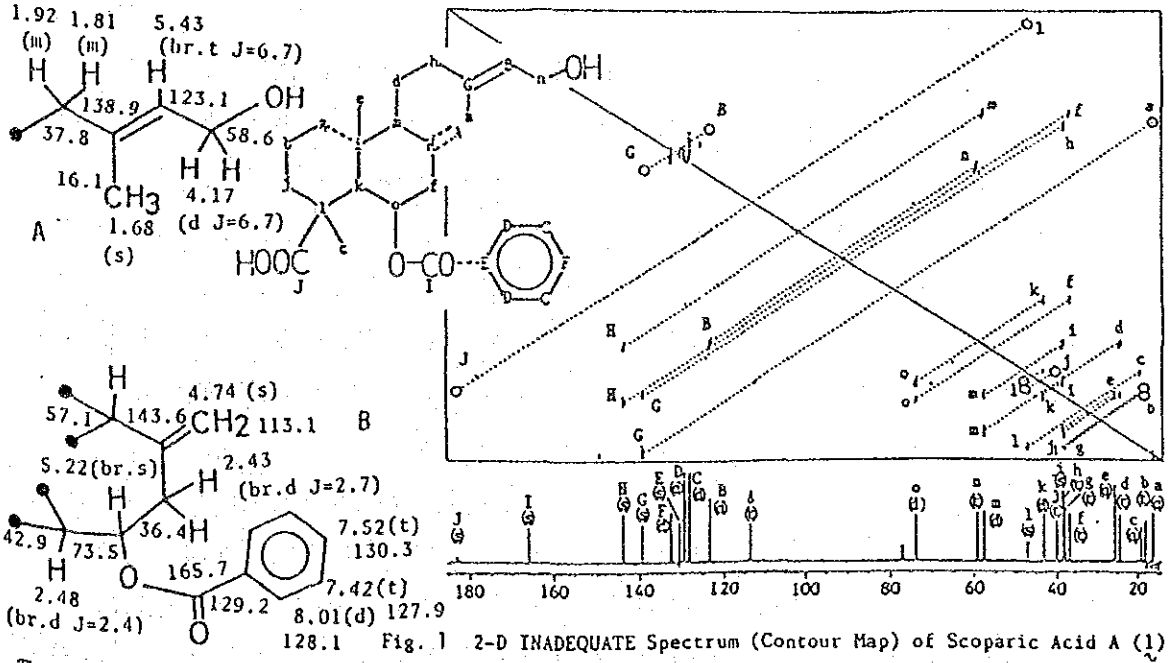


Fig. 1 2-D INADEQUATE Spectrum (Contour Map) of Scoparic Acid A (1)

The spectrum was measured on a JEOL GX-400 spectrometer, using 170 mg of the sample (40°C, 60hr run, J_{CC}=45Hz). Open circles indicate the expected carbon signals, which were not observed in this measurement, but were observed in other experiments.

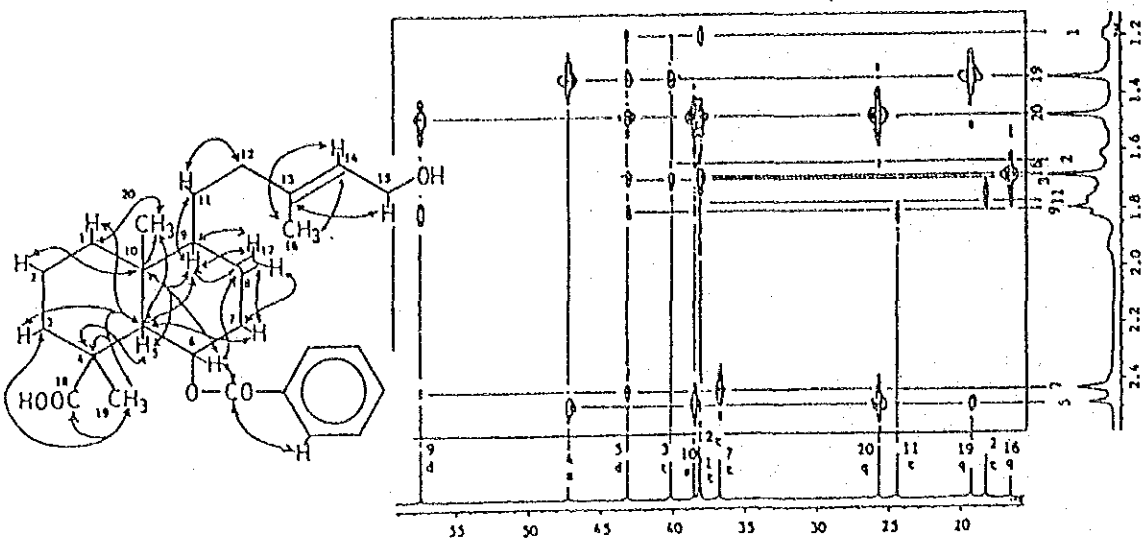


Fig. 2 ^{1}H-^{13}C Long-range COSY Spectrum of Scoparic Acid A in $CDCl_3$
(sample: 90 mg, 35°C, J_{CH}=10 Hz, 12 hr run)

($CHCl_3$) cm^{-1}: 3500、1710、1600、1580、UV (MeOH) nm(logε):230 (4.03)、266 (3.05)、278 (2.89)、の NMR[7] は $\underset{\sim}{1}$ と類似している．しかし、$\underset{\sim}{2}$ の ^{1}H-NMR には $\underset{\sim}{1}$ で認められたビニルメチル基 (δ1.68)、ヒドロキシメチル基(δ4.17) および δ5.43のオレフィンプロトンのシグナルが消失し、代わりに δ2.14に CH_3CO のシグナルが観察された．以上の事実より $\underset{\sim}{2}$ は $\underset{\sim}{1}$ の側鎖の二重結合が開裂して生じたケトン体であると推測された．$\underset{\sim}{2}$ の相対配置は結合定数と NOE 差スペクトルより $\underset{\sim}{1}$ と同じであると推定した．

c) Scoparic acid C ($\underset{\sim}{3}$)、無色粉末、$C_{26}H_{34}O_5$、$[\alpha]_D$ -13.9°($CHCl_3$)、IR ($CHCl_3$) cm^{-1}: 3500、1710、1700、1605、1590、UV (MeOH) nm (logε): 226 (4.08)、267 (2.96)、270 (2.94)、の NMR[8]も $\underset{\sim}{1}$ に似ているが、ビニルメチル基とヒドロキシメチル基のシグナルは消失し、代わりに新たなエキソメチレン基とアルデヒド基が観測された．また ^{1}H-^{1}H、および ^{1}H-^{13}C COSY の解析より、$\underset{\sim}{3}$ は $\underset{\sim}{1}$ より側鎖の炭素数が 1 個少なく、末端アルデヒド基を有し、これと共役したエキソメチレン基を有すると推定した．立体配位は NOE 差スペクトルの結果より $\underset{\sim}{3}$ 式で示されると推定した．

3．新規骨格をもつジテルペンカルボン酸類の構造

a) Scopadulcic acid A ($\underset{\sim}{4}$)、無色プリズム晶、mp 172-174°、$C_{27}H_{34}O_6$、$[\alpha]_D$ -5.7°(MeOH)、IR ($CHCl_3$) cm^{-1}: 3500、1710、1700、1600、1590、UV (MeOH) nm (logε): 229 (4.15)、275sh (2.98)、277 (3.02)、280 (2.94) はその ^{1}H-および ^{13}C-NMR[9] 上でカルボニル基 3 個、フェニル基

1 個、ヒドロキシメチル基 1 個、三級メチル基 2 個および四級 sp^3 炭素 4 個の存在を示した。さらに、^1H-^1H および ^1H-^{13}C COSY の詳細な解析により 4 は部分構造 C および D をもつことが示唆された(Fig. 3)。次いで 4 の ^1H-^{13}C long-range COSY より平面構造式を 4a と推定した(Fig. 4)。また 4 の相対配置は結合定数や NOE 差スペクトルの解析より 4b であると推定した(Fig. 5)。これは Scopadulcic acid A の単結晶 X 線回折により確証された(単斜晶形、$P2_1$、$z=2$、$R=0.063$)。Fig. 6 の ORTEP 図より C-5/C-10 および C-8/C-9 はトランスであり、C-4 のカルボキシル基、C-6 のベンゾイル基、C-10 のメチル基はいずれもアクシャル配位である。

b) Scopadulcic acid B (5)、無色プリズム晶、mp 228-232°、$C_{27}H_{34}O_5$、$[\alpha]_D$ -49.6° ($CHCl_3$) の IR および UV スペクトルは 4 と類似しているが、^1H および ^{13}C-NMR[10] には 4 に見られたヒドロキシメチル基に代わり三級メチル基が観察された。従って 5 の平面構造は 4 のヒドロキシメチル基がメチル基に置換されたものと推定された。5 の相対配置は NOE 差スペクトルにおいて Fig. 5 に示したような NOE が観察されたことより 5a であると推定した。

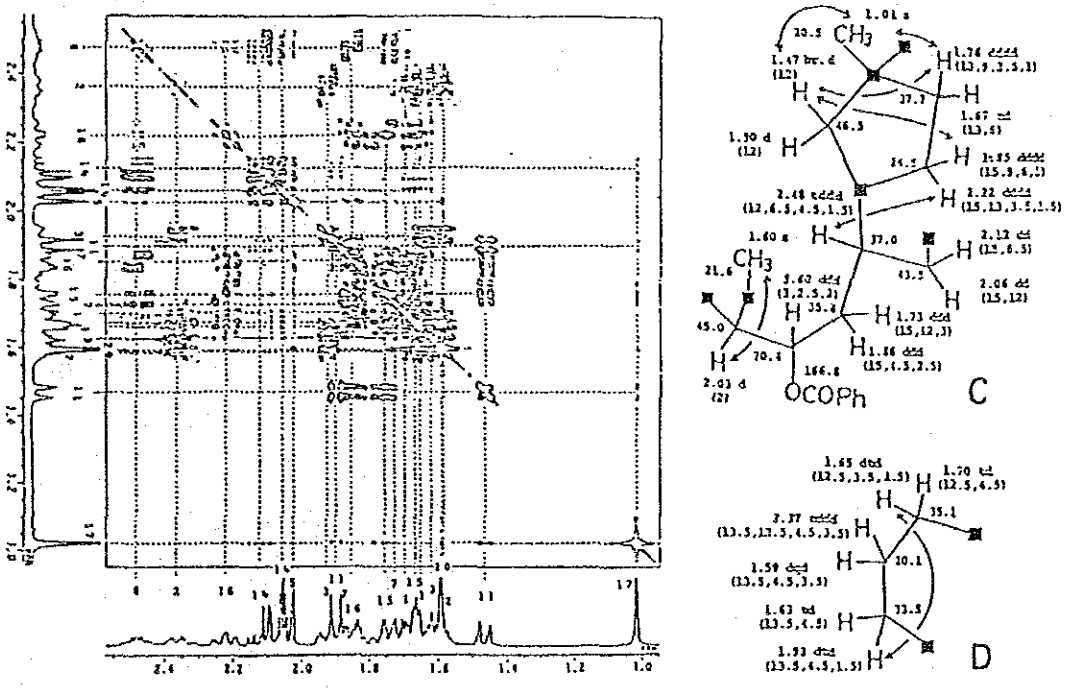


Fig. 3 Highly Resolved ^1H-^1H COSY Spectrum of Scopadulcic Acid A (4) in the High Field Region (in Acetone-d_6) and Partial Structures in 4
(⟿ : long-range coupling observed; coupling constants in parenthesis)

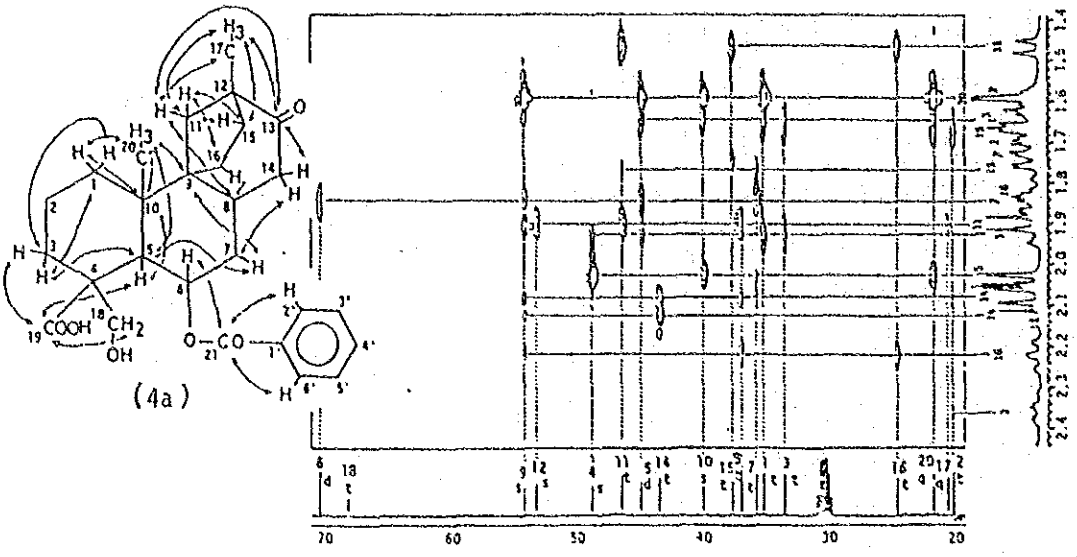


Fig. 4 ^{1}H-^{13}C Long-range COSY Spectrum of Scopadulcic Acid A in Acetone-d_6 (sample: 20 mg, 20°C, J_{CH}=10 Hz, 12 hr run)

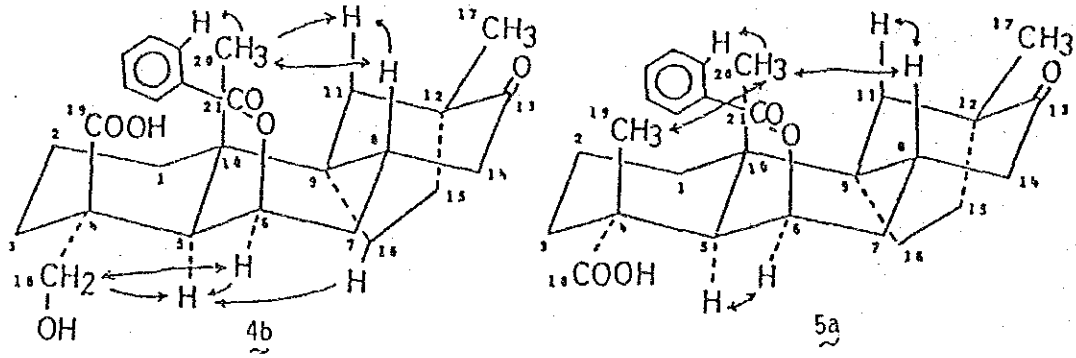
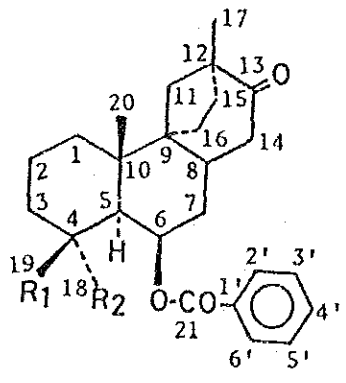


Fig. 5 NOE's Observed in Scopadulcic Acid A (4b) and B (5a)



4: R_1 = COOH, R_2 = CH_2OH
5: R_1 = CH_3, R_2 = COOH

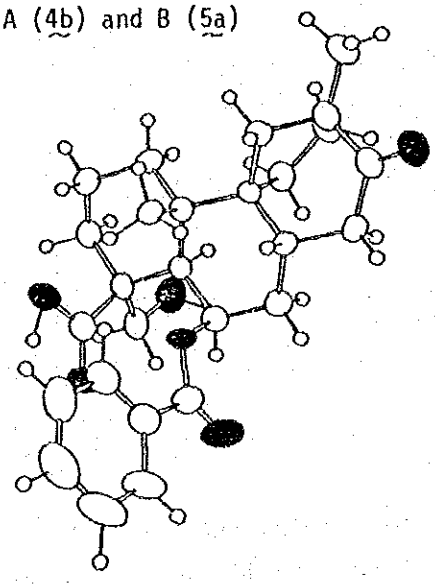


Fig. 6 ORTEP Drawing of Scopadulcic Acid A (4)

4．Scopadulcic acid A および B の絶対構造

Scopadulcic acid A および B の絶対構造は両者が CD スペクトルで正のコットン効果 (CD_{max} 4:$[\theta]_{297}$ +2130; 5:$[\theta]_{297}$ +2490) を示すことより、オクタント則を適用して各々 4b および 5a と決定した。

5．ジテルペンカルボン酸類の生物活性

得られたジテルペンカルボン酸類のうち Scoparic acid A (1)と C (3)は牛肝臓由来の β-glucuronidase に対し阻害作用を示した (5 μg/ml における阻害率、1: 72.0 %、3: 53.7 %)。また Scoparic acid B (2)、C (3)、Scopadulcic acid A (4) および B (5) はいずれも HeLa 細胞増殖抑制作用を示した (IC_{50} μg/ml、2: 0.94、3: 1.3、4: 0.78、5: 0.17)。さらに Scopadulcic acid B (5) には抗ウイルス作用 (HSV-1) が認められた (ED_{50} 0.012 μg/ml)。

謝辞： Scopadulcic acid A の X 線結晶解析を行って頂いた武田薬工中研、神谷和秀博士、和田喜一氏ならびに成分の HeLa 細胞に対する作用および抗ウイルス作用を調べて頂いた本学医学部、庭山清八郎教授、林京子博士に感謝致します。

References and notes

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2. M. C. Nath, Science and Culture, 7, 572 (1941-1942)
3. C. M. Chen and M. T. Chen, Phytochemistry, 15, 1997 (1976)
4. S. B. Mahato, M. C. Das and N. P. Sahn, Phytochemistry, 20, 171, (1981)
5. 1 の ^{13}C-NMR ($CDCl_3$)δ: 182.4 (s, 18-C), 165.7 (s, 21-C), 143.6 (s, 8-C), 138.9 (d, 13-C), 130.3 (d, 4′-C), 129.2 (s, 1′-C), 128.1 (d, 2′,6′-C), 127.9 (d, 3′,5′-C), 123.1 (d, 14-C), 113.1 (t, 17-C), 73.5 (d, 6-C), 58.6 (t, 15-C), 57.1 (d, 9-C), 46.8 (s, 4-C), 42.9 (d, 5-C), 39.7 (t, 3-C), 38.0 (s, 10-C), 37.8 (t, 12-C), 37.6 (t, 1-C), 36.4 (t, 7-C), 25.3 (q, 20-C), 24.0 (t, 11-C), 19.0 (q, 19-C), 17.9 (t, 2-C), 16.1 (q, 16-C)

6. C-9 位の配位は ^{1}H-NMR において 9-H のシグナルが他のシグナルと重なっており NMR スペクトルからは推定困難であるが、生合成的考察からβ配位と推定される。

7. 2 の ^{13}C-NMR ($CDCl_3$)δ: 209.1 (s, 13-C), 185.1 (s, 18-C), 167.1 (s, 21-C), 144.8 (s, 8-C), 132.8 (d, 4′-C), 130.8 (s, 1′-C), 129.9 (d, 2′,6′-C), 128.3 (d, 3′,5′-C), 113.1 (t, 17-C), 74.1 (d, 6-C), 56.8 (d, 9-C), 48.5 (s, 4-C), 43.8 (d, 5-C), 42.0 (t, 12-C), 40.2 (t, 3-C), 38.5 (s, 10-C), 38.1 (t, 1-C), 37.6 (t, 7-C), 30.1 (q, 16-C), 25.5 (q, 20-C), 20.1 (t, 2-C), 19.8 (q, 19-C), 18.8 (t, 2-C)

8. 3 の ^{13}C-NMR ($CDCl_3$)δ: 194.8 (d, 14-C), 185.3 (s, 18-C), 167.4 (s, 21-C), 150.4 (s, 13-C), 144.4 (s, 8-C), 134.2 (t, 16-C), 132.9 (d, 4′-C), 130.7 (s, 1′-C), 129.9 (d, 2′,6′-C), 128.3 (d, 3′,5′-C), 113.1 (t, 17-C), 74.1 (d, 6-C), 57.7 (d, 9-C), 48.2 (s, 4-C), 44.1 (d, 5-C), 40.2 (t, 3-C), 38.6 (s, 10-C), 38.2 (t, 1-C), 37.6 (t, 7-C), 26.7 (t, 12-C), 25.5 (q, 20-C), 24.3 (t, 11-C), 19.7 (q, 19-C), 18.8 (t, 2-C)

9. 4 の ^{13}C-NMR (acetone-d_6)δ: 212.8 (s, 13-C), 178.3 (s, 19-C), 166.8 (s, 21-C), 133.7 (d, 4′-C), 132.5 (s, 1′-C), 130.8 (d, 2′,6′-C), 129.5 (d, 3′,5′-C), 70.4 (d, 6-C), 68.2 (t, 18-C), 54.2 (s, 9-C), 53.2 (s, 12-C), 48.8 (s, 4-C), 46.5 (t, 11-C), 45.0 (d, 5-C), 43.5 (t, 14-C), 40.0 (s, 10-C), 37.7 (t, 15-C), 37.0 (d, 8-C), 35.8 (t, 7-C), 35.1 (t, 1-C), 33.5 (t, 3-C), 24.5 (t, 16-C), 21.6 (q, 20-C), 20.5 (q, 17-C), 20.1 (t, 2-C)

10. 5 の ^{13}C-NMR ($CDCl_3$)δ: 213.6 (s, 13-C), 184.2 (s, 18-C), 166.1 (s, 21-C), 133.4 (d, 4′-C), 130.5 (s, 1′-C), 129.6 (d, 2′,6′-C), 128.5 (d, 3′,5′-C), 72.9 (d, 6-C), 53.1 (s, 9-C), 52.3 (d, 15-C) 47.2 (s, 4-C), 45.1 (t, 11-C), 34.0 (t, 1-C), 23.7 (t, 16-C), 21.6 (q, 20-C), 19.7 (q, 17-C), 19.3 (q, 19-C), 18.0 (t, 2-C)

STRUCTURES OF NEW DITERPENOIDS FROM PARAGUAYAN CRUDE DRUG "TYPYCHA KURATU" (SCOPARIA DULCIS L.)

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In the course of searching of biologically active substances from Paraguayan medicinal plants, five new diterpenoids were isolated from Typychá kuratũ (whole plant of Scoparia dulcis L., Scrophuraliaceae). Their structures were elucidated mainly by means of 2-D NMR apectroscopy and NOE difference spectra. Three of them named scoparic acid A (1), B (2) and C (3) were labdane type diterpene acids. Other two compounds named scopadulcic acid acid A (4) and B (5) were diterpene acids with novel skeleton. The structure of scopadulcic acid A was confirmed by X-ray analysis. The absolute configuration of scopadulcic acid A and B were determined on the basis of CD spectral analysis.

日本生薬学会第34回年会　1987.10　大阪（吹田）

＜学会報告9＞

16-Ⅲ-B-8

パラグアイ産薬草の化学薬学的研究　第7報
Typychá kuratũ (*Scoparia dulcis* L.) に含まれるフラボノイドについて

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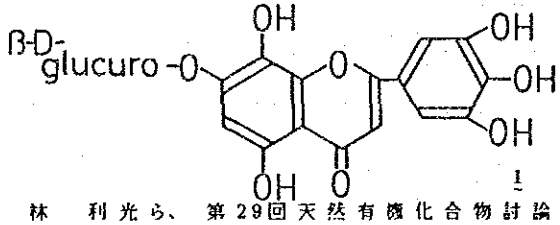
アスンシオン大・化学　L.H.Berganza

【目的】　先にパラグアイ産薬草、Typychá Kuratũ (*Scoparia dulcis* L.) の70%EtOH抽出エキスについて、β-glucuronidase(β-glu)阻害活性を指標にした分画を行い脂溶性画分より既知の friedelin, glutinol, α-amyrin, betulinic acidと共に、6種の新規ジテルペノイドを単離、報告した[1]。今回は比較的弱いβ-glu阻害活性を示した水溶性画分の成分分画を行い、得られた成分の構造研究を行った。また、現地の調査により *Scoparia dulcis* L. に形態的な多様性がみられたので、フラボノイド成分を指標にして成分の分布と地理的変異の有無を調べた。

【実験方法・結果】　乾燥したTypychá kuratũ の70%EtOH抽出エキスをchart 1に示したように分画し、水溶性画分(D)を得た。D(45g)を更にEt_2O, AcOEt-MeOH(9:1), AcOEt-MeOH(4:1)で順次振とう抽出し、各々D_1(1.65g), D_2(4.48g)およびD_3(28.77g)とした。D_1をポリアミドカラムに付し既に本植物より単離報告されている Scutellarein[2]の他に、apigenin, luteolin および *p*-coumaric acid が新たに単離された。一方、収量の多かったD_3からはポリアミドカラムクロマト、セルロースPLC、Sephadex LH-20 カラムクロマトを繰り返し行ったところ、vicenin-Ⅱ, linarin, vitexin, isovitexin, scutellarin[2] と化合物(1)が単離された。化合物(1)はUV吸収スペクトルとシフト試薬による挙動、^1H及び^{13}C-NMR スペクトルの解析により7-O-β-D-glucuronyl-8-hydroxy-tricetinと推定された。なお、D_2は目下分画中である。

今回単離されたフラボノイド類についてβ-glu に対する阻害作用を調べたところ，化合物(1)(IC_{50}:1.8×10^{-5}M) と isovitexin(IC_{50}:4.6×10^{-5}M)に阻害活性が認められた。パラグアイ南部の任意に選んだ6地点で採集した *Scoparia dulcis* の熱水抽出エキスについて2次元展開TLC（1次元目：*t*-BuOH-AcOH-H_2O, 3:1:1; 2次元目：15%AcOH)を行い，各TLCパターンの比較を行ったところ，形態的多様性にもかかわらずvicenin-Ⅱおよびsuctellarin が多量かつ普遍的に分布することが判明した。その他の微量成分は個体間或いは地域間に若干の変動が観察された。



1

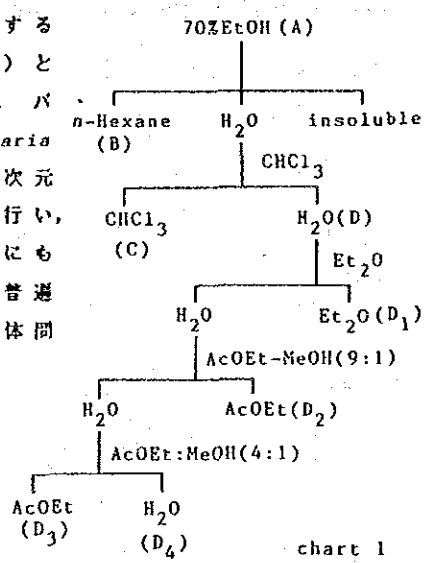


chart 1

1)　林　利光ら、第29回天然有機化合物討論会講演要旨集、札幌、1987、p544.
2)　本植物より既に単離報告されている。Ramesh *et al*. (1979), *Curr. Sci.* 48, 67.

日米合同薬学大会　1987.12

＜学会報告 10＞

Form A

JUC Pharm Sci '87

Abstract of Contributed Paper

このForm Aの送付先：〒150 東京都渋谷区渋谷 2－12－15 日本薬学会「日米合同薬学大会」事務局

CYTOTOXIC AND ANTIVIRALLY ACTIVE DITERPENOIDS FROM SCOPARIA DULCIS L.

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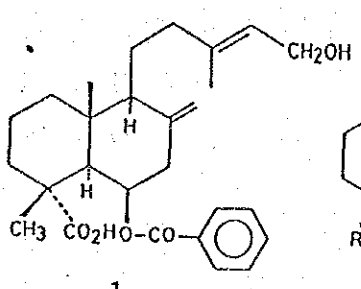
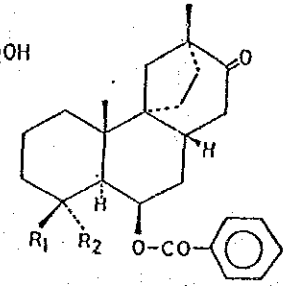
Three new diterpenoids named scoparic acid A (1), scopadulcic acid A (2) and scopadulcic acid B (3) were isolated from a Paraguayan crude drug "*Typychá kuratũ*" (whole plant of Scoparia dulcis L., Scrophulariaceae). Scopadulcic acid A exhibited cytotoxicities against KB and HeLa cells, while scopadulcic acid B showed antiviral activity against herpes simplex virus type 1 as well as cytotoxicity against HeLa cells. Scoparic acid A showed no significant activity in these bioassay systems.



1



2: R_1= COOH, R_2= CH_2OH

3: R_1= CH_3, R_2= COOH

日本薬学会第108年会　1988.4　広島
＜学会報告11＞

パラグァイ薬草の化学薬学的研究　第9報
利用状況に関するアンケート調査結果について

富山医薬大・薬　○林　利光、森田直賢
アスンシオン大・化　L.A.Palau, L.H.Berganza

パラグァイ薬草の利用状況を知る目的でアスンシオン市内及び近郊の一般住民に対しアンケート調査を実施したのでその結果について報告する。

アスンシオン第四市場で確認された薬草のうち267種について、利用経験の有無、利用部位、利用目的、利用方法等の回答を求めるアンケート用紙を1000名の住民に配布し、701名（男205名、女496名）から回答を得た。最も利用者が多かった薬草は消化、健胃等を目的とするBurrito（Minthostachys verticillata Epl, Labiatae）の葉で、469名もの回答があった。次いでAmbay（Cecropia pachystacha Trecの葉、咳、感冒、451名）、Cocu（Allophyllus guaranitica (Saint Hilaire) Radd var. guaraniticaの葉、清涼、肝炎、糖尿病、432名）の順であった。目的別に薬草を分けてみると、消化が40種で最も多く、次に清涼（34種）、利尿（28種）、堕胎（24種）、鎮咳（24種）の順であった。一方、利用方法としては①お茶を飲むように湯で温浸する②熱湯で煎じる③マテ茶とともに湯で温浸する④マテ茶とともに水で冷浸する方法が一般的であった。④はテレレと称するパラグァイ独特の飲み方で、暑い時の清涼飲料水代わりに広く利用されていることが分かった。

パラグァイの医療施設数は人口に比し、必ずしも少なすぎる状況ではないが、今回の調査の結果、薬草による民間療法が地方のみならず首都のアスンシオン市内においても住民の高い信頼度をもって利用されていることが明らかとなった。

日本生薬学会第33回年会　1986.10　埼玉

＜学会報告12＞

1A　10－2　パラグァイ薬草の化学薬学的研究　第3報

パラグァイ薬草の現況　その1　アスンシオン市場の薬草

富山医薬大・薬　○吉崎正雄、鈴木正一、清水岑夫、有澤宗久、林　利光
森田直賢
アスンシオン大　L. H. Berganza, E. Ferro, I. Basuldo

パラグァイ薬草の化学薬学的研究の一環として、パラグァイ国の民族が薬物として扱っている天然物に関し、アスンシオン市内の市場で1985年5月～1986年3月の間、約10回調査を行った結果を報告する。

調査した薬名はガラニー語名の薬物は約66％を占め、殆んど植物性の薬物で約280種、その内、生の形態は約70％、生薬は約30％であった。

薬物を薬用部位別にみると、地上部又は枝葉：約27％、全草：約17％、葉：約15％、根又は根茎：約11％、花：約10％、果実又は種子：約8％、皮：約8％、その他：約4％である。

効用は清涼薬、婦人病薬が目立ち、血液関係、糖尿関係、胃腸の鎮痛関係、肝臓、腎臓、心臓の疾患等に大別される。

使用法は水浸法と煎出法の2種が主である。多くは内用で、外用が10％位あった。

パラグァイでの薬草利用は治療以外に保健的な面での利用も多く、特にアスンシオン市内で見られる生の薬草は保健的な利用の様に思えた。パラグァイ特有の飲物にテレレがある。このテレレに用いる冷水に薬草を単品又は2～3種つぶしたものを入れて利用されている。

今回総論的に述べる。

添 付 論 文

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② Chem. Pharm. Bull. 37, 2531 (1989)

③ Chem. Pharm. Bull. 36, 4447 (1988)

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⑤ J. Nat. Prod. 51 (2), 357 (1988)

⑥ 生薬学雑誌 43 (1), 78 (198)

⑦ Plomta Medica 53 (4), 394 (1987)

⑧ J. Nat. Prod. 50 (6), 394 (1987)

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<添付論文1>

[Chem. Pharm. Bull. 35(3)1234—1237(1987)]

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).

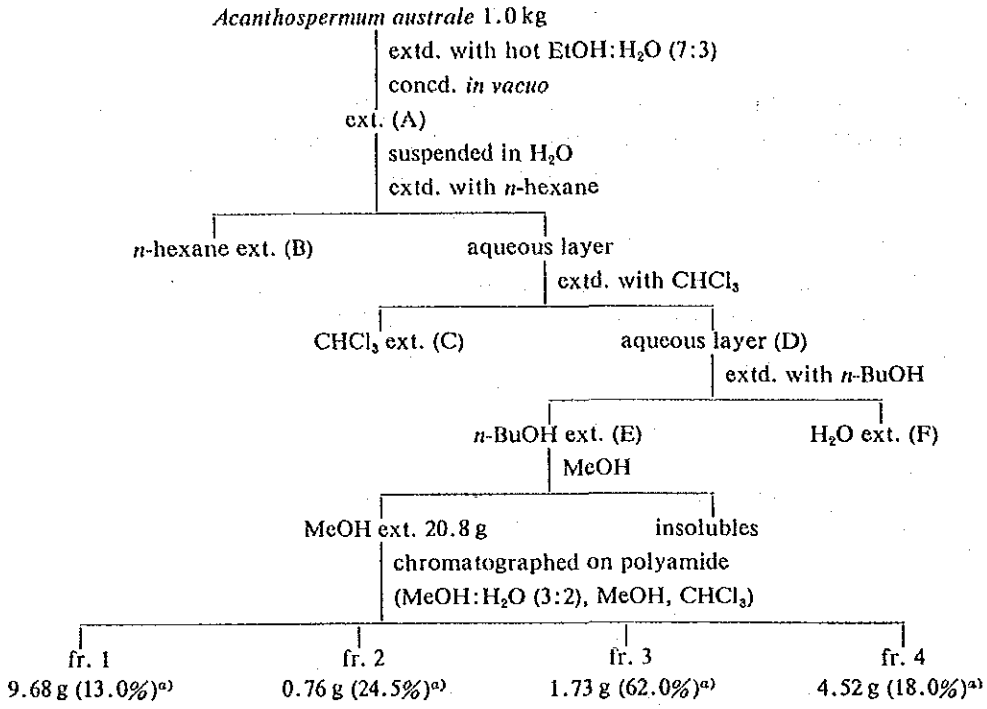

Fig. 1. Fractionation of Biologically Active Constituents of *Acanthospermum australe*

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

TABLE 1. 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

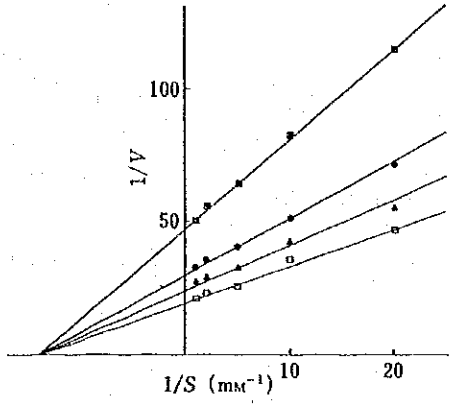

Fig. 2. Lineweaver–Burk Plots of Lens AR Activity

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 ($IC_{50} = 1.0 \times 10^{-7}$ M) among compounds **1**—**6** and was about 18 times more potent than quercitrin ($IC_{50} = 1.8 \times 10^{-6}$ M) (Table I).

According to Okuda *et al.*,[7] axillarin and LAR1 **1** are the most potent inhibitors of aldose reductase known so far ($IC_{50} = 5.2 \times 10^{-8}$ 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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<添付論文2>

Studies on Aldose Reductase Inhibitors from Natural Products. II.[1] Active Components of a Paraguayan Crude Drug "Para-parai mi," *Phyllanthus niruri*

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Aldose reductase (AR) inhibitory activity-directed fractionation of the 70% ethanolic extract of Para-parai mi, *Phyllanthus niruri*, has led to the isolation of three active components, ellagic acid (1), brevifolin carboxylic acid (4) and ethyl brevifolin carboxylate (5). Among them, 1 showed the highest inhibitory activity, being about 6 times more potent than quercitrin, which is a known natural inhibitor of AR.

Keywords *Phyllanthus niruri*; Euphorbiaceae; aldose reductase inhibitor; ellagic acid; brevifolin carboxylic acid; ethyl brevifolin carboxylate

In screening tests for biological activities of medicinal plants in Paraguay, Para-parai mi, the whole plant of *Phyllanthus niruri* L. (Euphorbiaceae), which has traditionally been used for dissolution of calculus and as a diuretic in Paraguay, was found to have inhibitory activities against angiotensin converting enzyme (ACE) and rat lens aldose reductase (AR), which plays a significant role in the reduction of aldose to alditol under abnormal conditions such as diabetes. We previously reported the isolation of geraniin as an ACE inhibitor.[3] In this paper, we report the isolation and identification of the active components inhibiting rat lens AR.

The 70% EtOH extract (A) of Para-parai mi which showed high inhibitory activity towards crude rat lens AR ($IC_{50} = 1.0 \times 10^{-6}$ g/ml), was partitioned between *n*-hexane and water to afford the *n*-hexane extract (B), water layer and a precipitate (C) insoluble in both solutions. The water layer was further extracted with $CHCl_3$ and *n*-BuOH successively to afford the $CHCl_3$ extract (D), *n*-BuOH extract (E) and a residue (F) (Fig. 1). The precipitate C, which was most active (Table I), was applied to a column of Sephadex LH-20 to give five compounds 1—5. Compounds 1 and 2 were identified as ellagic acid and gallic acid by comparing them with the respective authentic samples. Compound 3 gave 1, 2 and D-glucose on hydrolysis with 2 N HCl suggesting it to be corilagin[4] and this identification was cofirmed by direct comparison with an authentic sample.

Compound 4, mp > 250 °C, gave a positive color reaction to $FeCl_3$ and was also considered to be an ellagic acid analogue from the spectral data. Compound 4 showed a molecular ion peak at *m/z* 292 in the mass spectrum (MS), and its infrared (IR) spectrum suggested the presence of carboxylic acid and α,β-unsaturated lactone moieties (1710, 1680 and 1620 cm^{-1}). Decarboxylation of 4 in a fused glass tube at 140 °C gave brevifolin[4] which was identified by comparison with an authentic sample. As a result, 4 was deduced to be brevifolin carboxylic acid.[5] Compound 4 was methylated with diazomethane to give trimethyl brevifolin carboxylic acid methyl ester, whose melting point (164—166 °C) coincided with the reported value.[5]

Compound 5, yellowish powder, showed a molecular ion peak at *m/z* 320 in the MS ($-C_2H_5$ unit more than 4) and absorption bands at 1720, 1690, 1630 and 1600 cm^{-1} in the IR spectrum, suggesting it to be the ethyl ester of 4. In addition, the ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum of 5 (given in the Experimental section) was consistent with this structure.

Compounds 1—4 have generally been regarded as hydrolysis products of geraniin,[6-9] which was isolated as an ACE inhibitor from this plant[3] and is distributed widely in plants, especially in Euphorbiaceae.[9] This is the first report of the natural occurrence of 5, but the possibility remains that it might be an artifact derived from geraniin. No other active constituent was found in the extract E.

Inhibitory Effect on Crude Rat Lens AR The isolated compounds 1—5 and geraniin were tested for AR-inhibitory activity (Table I). (Geraniin showed no effect and has not been included in Table I.) Among them, 1 exhibited the highest activity ($IC_{50} = 2.0 \times 10^{-7}$ M), being more potent than quercitrin ($IC_{50} = 1.2 \times 10^{-6}$ M), tested as a reference.

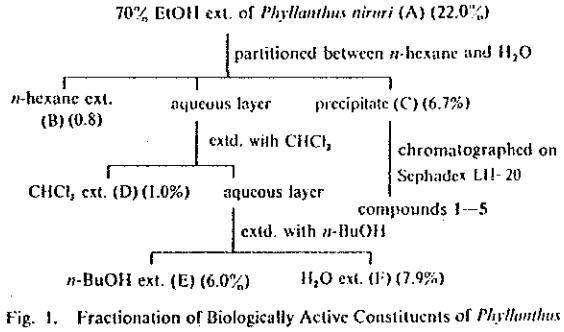

Fig. 1. Fractionation of Biologically Active Constituents of *Phyllanthus niruri*

Values in parenthesis indicate the yield (%) from dried material.

TABLE I. Inhibition of Crude Rat Lens AR by Extracts from *Phyllanthus niruri* and Compound 1—5

Extract	IC_{50} (μg)	Compound	IC_{50} (μM)
A	1.0	1	0.2
B	>20	2	—
C	0.3	3	—
D	11.0	4	2.4
E	1.2	5	5.0
F	3.8	Quercitrin[a]	1.2

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

Compounds 4 and 5 also showed some effect.

In order to determine the type of inhibition, the kinetics of inhibition of AR by 1 were plotted according to Lineweaver and Burk and 1 was found to be a non-competitive inhibitor at the concentration of IC_{50} (2.0×10^{-7} M), as was seen in the cases of quercitrin and 5,7,4'-trihydroxy-3,6-dimethoxyflavone[1] reported by us.

Results and Discussion

It is currently expected that AR inhibitors will play an important role in the management of diabetic complications such as cataract. Carboxylic acid, flavonoid and hydantoin analogues, *etc.*, have hitherto been examined as AR-inhibitory drugs, but this is the first report of ellagic acid and tannin-related compounds as AR inhibitors. Tannins have many kinds of biological activities, but geraniin, a typical tannin, showed no effect on AR, whereas ellagic acid, considered to be a hydrolysis product of geraniin, showed a strong activity. We are now investigating the AR-inhibitory effect of ellagic acid-related compounds and will report the results in the future.

Experimental

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Spectral data were obtained as follows: IR spectra with a Hitachi 260-0611 spectrophotometer; ultraviolet (UV) spectra with a Hitachi 270S spectrophotometer; MS with a JEOL JMS-D 200 spectrometer (70 eV); ^{13}C-NMR spectra with a Varian XL-200 (50.3 MHz) spectrometer in dimethyl sulfoxide (DMSO)-d_6. Chemical shifts are given in δ(ppm) values referred to internal tetramethylsilane.

Plant Materials Para-parai mi was purchased from local dealers in Asuncion, Paraguay, and identified as *Phyllanthus niruri* L. Voucher specimens are on deposit at both the Institute of Toyama Medical and Pharmaceutical University and Asuncion 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] and showed a specific activity of 22 units/mg. One unit was defined as the amount catalyzing the oxidation of 1 μmol of reduced nicotinamide adenine dinucleotide phosphate per minute. Inhibitory effects of the extract and isolated compounds on AR were assayed by the method previously reported.[1,11] Samples were dissolved in DMSO which was found to have no effect on the enzyme activity at below 0.1% concentration.

Extraction and Isolation Dried powder of the material (3.7 kg) was extracted with hot 70% EtOH (1 h × 3) to yield 796 g of the dried extract A. The extract A (786 g) was partitioned between *n*-hexane (3.9 l × 5) and distilled water (3.9 l) to afford an *n*-hexane extract B and an insoluble precipitate C (243 g). The aqueous layer was extracted with $CHCl_3$ and *n*-BuOH successively to yield $CHCl_3$, *n*-BuOH and H_2O extracts, D, E and F. The biologically active C (32 g) was chromatographed on a Sephadex LH-20 column eluting first with 70% MeOH and then with increasing percentages of MeOH to yield compounds 1 (3.6 g), 2 (10 mg), 3 (26 mg), 4 (10 mg) and 5 (25 mg). Ellagic acid (1), mp > 300 °C, gallic acid (2), mp 240—241 °C (dec.) and corilagin (3), mp 193—195 °C (dec.) were identified by direct comparisons with the respective authentic samples (IR, paper partition chromatography).

Brevifolin Carboxylic Acid (4) Yellowish powder, mp > 250 °C (dec. not sharp) (MeOH), yellow-green to $FeCl_3$ reagent. MS *m/z*: 292 (M^+), 246 (M − COOH − H), 218 (246 − CO), 204, 190, 162. *Anal.* Calcd for $C_{13}H_8O_8$: C, 53.44; H, 2.76. Found: C, 53.21; H, 2.90. IR ν_{max}^{KBr} cm^{-1}: 3200, 1710, 1680, 1620, 1590. UV λ_{max}^{MeOH} nm (log ε): 365 (3.79), 350 (3.81), 280 (4.13). A solution of 4 (3 mg) in distilled H_2O (2 ml) was heated in a fused glass tube at 140 °C for 1 h, and the precipitate was recrystallized from MeOH to afford brevifolin (2 mg), which was identified by comparison with an authentic sample (IR). 4 gave trimethyl brevifolin carboxylic acid methyl ester, mp 164—165 °C, with CH_2N_2. (Spectral data could not be obtained due to an insufficient amount of sample).

Ethyl Brevifolin Carboxylate (5) Yellowish powder, mp > 250 °C (dec.) (MeOH), yellow-green to $FeCl_3$ reagent. MS *m/z*: 320 (M^+), 274 (M − OC_2H_5 − H), 246, 218 (246 − CO), 204, 190, 162. *Anal.* Calcd for $C_{15}H_{12}O_8$: C, 56.26; H, 3.78. Found: C, 56.02; H, 3.89. IR ν_{max}^{KBr} cm^{-1}: 3150, 1720, 1690, 1630, 1600. UV λ_{max}^{EtOH} nm (log ε): 365 (3.94), 350 (3.96), 276 (4.34). ^{13}C-NMR (50.3 MHz, DMSO-d_6) δ: 192.9, 171.9, 160.0, 149.5, 145.6, 143.5, 140.1, 138.4, 138.3, 114.8, 112.8, 107.9, 60.5, 36.9, 13.8.

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<添付論文3>

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Anti-inflammatory Constituents of Topically Applied Crude Drugs. III.[1] Constituents and Anti-inflammatory Effect of Paraguayan Crude Drug "Tamandá cuná" (*Catasetum barbatum* LINDLE)[2]

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The 70% EtOH extract of the aerial parts of *Catasetum barbatum* LINDLE showed inhibitory effects on carrageenin-induced paw edema when topically applied to rats and on histamine-induced contraction in guinea pig ileum.

Four compounds including a new phenanthrene, 2,7-dihydroxy-3,4,8-trimethoxyphenanthrene (**2**), were isolated from the active fraction. 2,7-Dihydroxy-3,4-dimethoxyphenanthrene (**1**) showed inhibitory effects in both biological assays.

Keywords——anti-inflammatory effect; *Catasetum barbatum*; Orchidaceae; 2,7-dihydroxy-3,4-dimethoxyphenanthrene; 2,7-dihydroxy-3,4,8-trimethoxyphenanthrene; carrageenin edema; H_1-receptor

In the course of a search for biologically active substances from Paraguayan medicinal plants, the 70% EtOH extract of the aerial parts of *Catasetum barbatum* LINDLE (Orchidaceae), "Tamandá cuná" in Paraguay, was found to have anti-inflammatory activity when topically applied. Tamandá cuná is a folk medicine used for the treatment of asthma, lumbago, *etc.* in Paraguay, and little is known about the chemical constituents.

In this paper, we report the separation and identification of some chemical constituents of the active fraction along with their anti-inflammatory activity in the carrageenin-induced paw edema (CPE) test and inhibitory effect on histamine-induced contraction in guinea pig ileum (HCI).

The 70% EtOH extract (fr. A) was suspended in water and extracted with Et_2O under acidic conditions (pH 3) to give the Et_2O-soluble fraction (fr. B) and H_2O-soluble fraction (fr. C). The active fr. B was further fractionated as shown in Chart 1 to afford acidic (fr. D), weakly acidic (fr. E) and neutral fractions (fr. F).

In the CPE test with topical application[1] of frs. A—F, frs. A, B and E showed inhibitory activity at an early time after carrageenin injection (Table I).

Thus, we examined the inhibitory effect on the contraction in guinea pig ileum induced by histamine (considered to be a chemical mediator at the early stage of acute inflammation), and the result (shown in Table II) corresponded well with that of the CPE test.

Active fr. E was subjected to column chromatography on silica gel with a solvent system of $CHCl_3$–MeOH (gradient), with monitoring by thin layer chromatography (TLC), to give four fractions (frs. 1—4). Fractions 1 and 2 showed inhibitory effects on HCI and were further

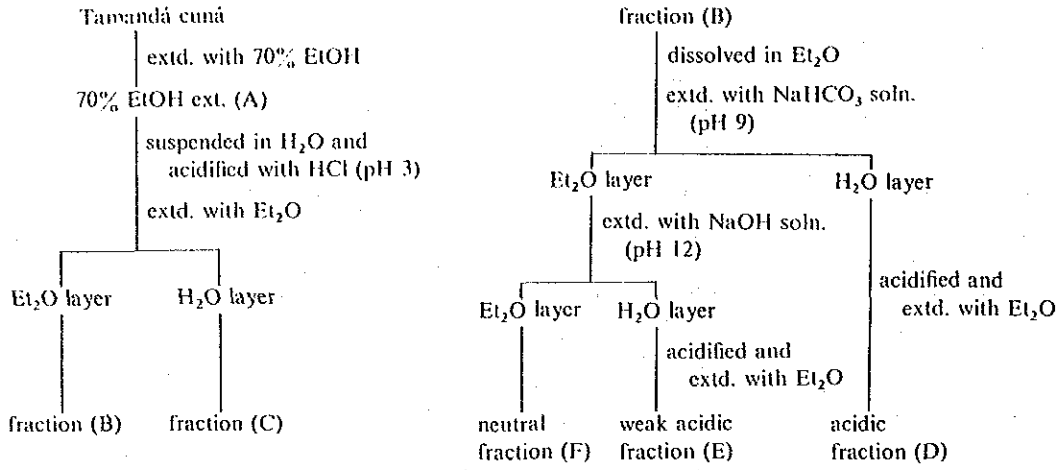


Chart 1. Extraction and Fractionation of Aerial Parts of Tamandá cuná

TABLE I. Inhibitory Effects of Frs. A—F and Compound 1 on Carrageenin Paw Edema in Rats[a]

Fraction and compound	Dose (mg/site × 4)	Inhibition of swelling (%)			
		1 h	2 h	3 h	4 h[b]
A	5	30.7[d]	22.8[d]	17.4	—
B	5	57.9[e]	20.3	24.1[d]	17.1
C	5	—[c]	—	—	—
D	5	—	—	14.3	18.6
E	5	43.5[d]	21.8[d]	29.7[d]	32.5[d]
F	5	—	17.2	14.2	18.6
Indomethacin	0.5	—	15.8	37.3[d]	33.3[d]
1	4	34.6[d]	33.4[d]	31.1[d]	36.8[e]

a) $n=4$ or 5. *b*) Time after carrageenin injection. *c*) No effect (less than 10%). *d*) $p<0.05$. *e*) $p<0.01$.

TABLE II. Inhibitory Effects of Frs. A—F and Compounds 1—4 on Histamine (10^{-7} g/ml)-Induced Contraction in Guinea Pig Ileum

Fraction	IC_{50} (g/ml)	Compound	IC_{50} (g/ml)
A	4.8×10^{-5}	1	5.4×10^{-6}
B	3.0×10^{-5}	2	9.0×10^{-6}
C	$>10^{-4}$	3	1.5×10^{-5}
D	$>10^{-4}$	4	2.0×10^{-5}
E	1.1×10^{-5}	Diphenhydramine·HCl	1.2×10^{-8}
F	$>10^{-4}$		

separated and purified by repeated column chromatography to yield compounds **1**—**4**.

Compound **1**, colorless needles, mp 157—158 °C, $C_{16}H_{14}O_4$, showed a positive reaction with the ferric chloride reagent. It showed a typical ultraviolet (UV) spectrum[3] of phenanthrene (see the experimental section), and gave a diacetate (**1a**) with acetic anhydride and pyridine. In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, **1** showed the presence of two phenolic methoxyl groups and six aromatic protons. The positions of the substituents were presumed to be C-2, 3, 4 and 7 from the chemical shifts and splitting patterns of aromatic protons (1H singlet, 3H ABX-type and 2H singlet) in the ^{1}H-NMR spectrum.

On the basis of the spectroscopic evidence, compound **1** was finally determined to be 2,7-dihydroxy-3,4-dimethoxyphenanthrene by comparisons of the ^{1}H-NMR and UV spectra of **1** and **1a** with those of an authentic sample.[4] This is the first isolation of this compound from the genus Catasetum.

Compound **2**, colorless needles, mp 197—199 °C, showed a positive ferric chloride reaction and its molecular formula was determined to be $C_{17}H_{16}O_5$ from the high-resolution mass spectrum (MS). The UV spectrum showed very similar absorption bands to those of **1**, indicative of a phenanthrene derivative. Compound **2** gave a diacetate (**2a**) and the ^{1}H-NMR

spectrum showed the presence of three phenolic methoxyl and five aromatic protons, giving two pairs of AB-type signals and a singlet signal, respectively (Table III). One pair of signals (δ 9.10 and 7.25) was assigned to H-5 and H-6, and the other pair (δ 7.90 and 7.58) to H-9 and H-10. Comparing the signals due to H-6 in **1** and **2**, the latter was observed as a doublet owing to only *ortho* coupling in **2**, indicating the absence of H-8 (the former was a doublet of doublets). In addition, the signals due to H-9 and H-10 in **2** each appeared as a doublet, observed only in the case of 1- or 8-substituted phenanthrene,[4] suggesting the presence of a substituent at the 8 position. A 2H singlet was seen in the case of **1** which has no substituent at the 1 or 8 position. Therefore, the positions of substituents should be C-2, C-3, C-4, C-7 and C-8. The locations of hydroxyl groups were concluded to be C-2 and C-7 from the acetylation shifts of H-1 and H-6 (Δ 0.36 and 0.17 ppm) compared with those of **1** (Δ 0.36 and 0.14 ppm) and 8-hydroxy-2,3,4,7-tetramethoxyphenanthrene (**5**)[5] (Δ 0.2 and 0.04 ppm) (Table III).

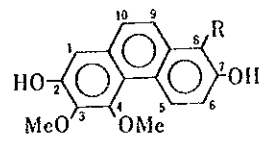
On the basis of these findings, compound **2** was determined to be 2,7-dihydroxy-3,4,8-trimethoxyphenanthrene, which is a previously unknown phenanthrene.

Compound **3**, colorless columns, showed the molecular formula $C_{16}H_{16}O_4$, having one mol of hydrogen more than **1**. The UV spectrum showed the absorption maximum at 281 nm. **3** gave a diacetate (**3a**) and the ^{1}H-NMR spectrum indicated the presence of two phenolic methoxyl and four aromatic protons as ABX-type signals and a singlet signal, besides a four-proton singlet at δ 2.63, typical of a 9,10-dihydrophenanthrene. From these findings, **3** was

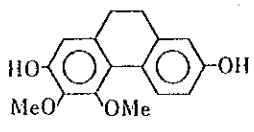
TABLE III. ^{1}H-NMR Spectral Data for **1**, **1a**, **2**, **2a**, **5** and **5a**, δ (ppm) from TMS (J=Hz)

Proton	1[a]	1a[a]	2[a]	2a[a]	5[b]	5a[b]
1	7.16 (s)	7.52 (s)	7.17 (s)	7.53 (s)	7.00 (s)	7.20 (s)
5	9.35 (d, J=9.2)	9.60 (d, J=9.2)	9.10 (d, J=9.7)	9.37 (d, J=9.7)	8.98 (d, J=9)	9.40 (d, J=10)
6	7.31 (dd, J=9.2, 2.4)	7.45 (dd, J=9.2, 2.4)	7.25 (d, J=9.7)	7.42 (d, J=9.7)	7.29 (d, J=9)	7.33 (d, J=10)
8	7.17 (d, J=2.4)	7.69 (d, J=2.4)	—	—	—	—
9	7.51 (2H, s)	7.75 (2H, s)	7.90 (d, J=9.2)	8.08 (d, J=9.2)	8.00 (d, J=9)	7.60 (2H, s)
10			7.58 (d, J=9.2)	7.77 (d, J=9.2)	7.84 (d, J=9)	
OMe	4.01 (s)	4.01 (s)	4.00 (s)	4.02 (6H, s)	3.99 (6H, s)	4.00 (s)
	3.97 (s)	3.99 (s)	3.96 (s)	3.97 (s)	3.98 (6H, s)	3.97 (s)
			3.93 (s)			3.95 (6H, s)
OH/OAc	8.44 (2H, br s)	2.37 (s)	8.30 (2H, br s)	2.39 (s)	5.98 (br)	2.45 (s)
		2.34 (s)		2.37 (s)		

a) In acetone-d_6. *b*) In $CDCl_3$. Compound **5**, 8-hydroxy-2,3,4,7-tetramethoxyphenanthrene; **5a**, monoacetate of **5**, values in literature.



1 : R=H
2 : R=OMe



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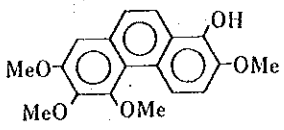

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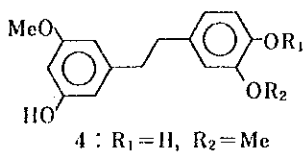

4 : R_1=H, R_2=Me
6 : R_1=Me, R_2=H

Chart 2

considered to be the 9,10-dihydrogenated derivative of **1**.

To confirm the structure, **3a** was dehydrogenated with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) as described in the experimental section, to give the corresponding derivative, which was found to be identical with **1a** in terms of the ^{1}H-NMR spectrum. Compound **3** was thus concluded to be 2,7-dihydroxy-3,4-dimethoxy-9,10-dihydrophenanthrene, which has recently been isolated from *Eria carinata* and *E. stricta*.[6]

Compound **4**, a colorless oil, $C_{16}H_{18}O_4$, gave the signals of six aromatic, two phenolic methoxyl, two hydroxyl (disappeared on D_2O treatment), and four equivalent benzylic protons in the ^{1}H-NMR spectrum. The MS of **4** showed a molecular ion peak at *m/z* 274 and a base peak at *m/z* 137, arising from the tropilium ion formed by benzylic cleavage of the biphenylethane containing one hydroxyl and one methoxyl group in both aromatic rings. The structure of **4** was thus considered to be 3,4′-dihydroxy-5,5′-dimethoxydihydrostilbene (**4**) or 3,5′-dihydroxy-5,4′-dimethoxydihydrostilbene (**6**) from the splitting pattern of the aromatic protons in the ^{1}H-NMR spectrum (3H, each *meta*-coupled, and 3H, ABX-type). Crombie and Jamieson[7] reported **4** as an oil and **6** as colorless needles (mp 132—133 °C); the spectral data of the former were in agreement with those of **4** isolated by us (oil).

Finally, compound **4** was identified as 3,4′-dihydroxy-5,5′-dimethoxydihydrostilbene by comparison of the ^{1}H- and ^{13}C-NMR spectra with those of an authentic sample, and was confirmed to be different from **6**, kindly provided by Dr. Crombie, in terms of physical and spectral data.

Recently, Juneja *et al.*[8] reported the isolation of gigantol as crystals (mp 94—95 °C) from *Cymbidium giganteum* and proposed the structure **4**, but the physical and spectral data are different from those of **4** isolated by us and synthesized by Crombie and Jamieson. 'Gigantol' is rather similar to **6**, but the structure is still in question.

Biological Activities of Compounds 1—4

Compounds **1**—**4** isolated from *C. barbatum* LINDLE in the present study were tested for inhibitory effect on HCl. Among these four compounds, **1** showed the strongest activity and the other compounds also showed activity as strong as that of fr. E (Table II). We also examined the mode of action of compound **1** against H_1-receptor. As shown in Fig. 1, the histamine-induced contraction did not reach the control level when compound **1** was used at 3×10^{-6} g/ml, and the tendency increased with increasing concentration of compound **1**. From these results, compound **1** was considered to be a specific non-competitive inhibitor of H_1-receptor.

Further, compound **1** showed activity in the CPE test on topical application throughout the test period, being significantly different from the control (Table I). The other three

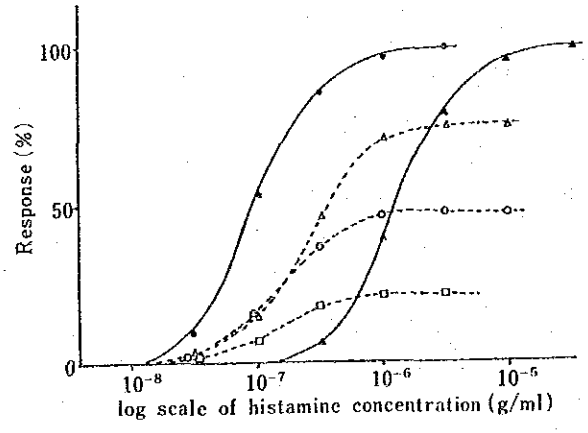

Fig. 1. Dose–Response Curve for Compound **1** on Histamine-Induced Contraction in Guinea Pig Ileum

●—●, control; ▲—▲, diphenhydramine 3×10^{-8} g/ml; △---△, compound **1** 3×10^{-6} g/ml; ○---○, compound **1** 6×10^{-6} g/ml; □---□, compound **1** 1×10^{-5} g/ml.

compounds could not be examined with this test owing to limited sample availability, but might show anti-inflammatory activity in view of the resemblance in chemical structure. In addition, it is of interest that **4** was isolated from the anti-inflammatory active fraction, considering that Goda *et al.* reported that a dihydrostilbene derivative was an inhibitor of prostaglandin and thromboxane synthetase.[9]

Experimental

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Spectral data were obtained as follows: infrared (IR) spectra with a Hitachi 260-0611 spectrophotometer; UV spectra with a Hitachi 270 S spectrophotometer; MS with a JEOL JMS-D 200 spectrometer; ^{1}H-NMR spectra with a JEOL FX 90Q (90 MHz) spectrometer; ^{13}C-NMR spectra with a JEOL FX 90Q (22.5 MHz) spectrometer. Chemical shifts are given in δ (ppm) values referred to internal tetramethylsilane (TMS). TLC was carried out on precoated Kieselgel 60 F_{254} plates (Merck). The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet and br=broad.

Plant Materials——Aerial parts of *Catasetum barbatum* LINDLE were collected at Departomento Presidente Hayes in Chaco, Paraguay, in July 1985 and 1986. Voucher speciments are on deposit in the institute of Asuncion University.

Bioassay——Anti-inflammatory activity by topical application toward CPE and inhibitory effect on HCl were assessed as described in a previous report.[10]

Extraction and Fractionation——Fresh aerial parts of *Catasetum barbatum* LINDLE (6.8 kg) were extracted with hot 70% EtOH to give the extract (78 g) (fr. A). Fr. A was suspended in water and extracted with Et_2O under acidic conditions (pH 3) to afford the Et_2O-soluble fraction (fr. B, 14.2 g) and H_2O-soluble fraction (fr. C, 62.0 g).

Fr. B (active in the CPE test) was dissolved in Et_2O and extracted first with $NaHCO_3$ solution (pH 9) and next with NaOH solution (pH 12), followed by extraction with Et_2O again after neutralization of each alkaline solution to afford acidic (fr. D, 1.2 g) and weakly acidic fractions (fr. E, 3.6 g), and the neutral fraction (fr. F, 9.2 g) from the alkali insoluble Et_2O layer.

Constituents of the Active Fraction (Fr. E)——Fr. E (3.1 g), showing activity in the CPE test and an inhibitory effect on HCl, was chromatographed on silica gel to give fr. 1 (0.50 g), fr. 2 (0.52 g), fr. 3 (1.1 g) and fr. 4 (0.98 g) from the eluates with a $CHCl_3$–MeOH gradient system.

Fr. 1 and fr. 2 showing an inhibitory effect in the HCl test were repeatedly chromatographed together on silica gel with *n*-hexane–AcOEt (5 : 1—3 : 1) to give **1** (155 mg), **2** (73 mg), **3** (41 mg) and **4** (21 mg).

2,7-Dihydroxy-3,4-dimethoxyphenanthrene (1)——Colorless needles, mp 157—158 °C ($CHCl_3$–MeOH). *Anal.* Calcd for $C_{16}H_{14}O_4$: C, 71.10; H, 5.22. Found: C, 70.68; H, 5.19. UV λ_{max}^{MeOH} nm (log ε): 364 (3.18), 346 (3.14), 303 (3.82), 292 (4.08), 283 (4.15), 258 (4.85), 229 (4.13). MS *m/z*: 270.0926 (M^+, Calcd for $C_{16}H_{14}O_4$: 270.0891), 255, 212. IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1610, 1575 (phenyl nucleus). ^{1}H-NMR (in acetone-d_6): Table III.

2,7-Diacetoxy-3,4-dimethoxyphenanthrene (1a)——**1** was acetylated with acetic anhydride and pyridine at room temperature overnight and the mixture was added dropwise to iced water to yield **1a**, colorless needles, mp 157—160 °C (*n*-hexane–AcOEt). MS *m/z*: 354 (M^+), 312 ($M^+ - CH_2CO$), 270 ($M^+ - 2CH_2CO$, base peak), 255, 212. IR ν_{max}^{KBr} cm^{-1}: 1760, 1620, 1560. ^{1}H-NMR (in acetone-d_6): Table III.

2,7-Dihydroxy-3,4,8-trimethoxyphenanthrene (2)——Colorless needles, mp 197—199 °C ($CHCl_3$–AcOEt). UV λ_{max}^{MeOH} nm (log ε): 367 (3.36), 349 (3.31), 331 (3.12), 307 (4.15), 295 (4.27), 286 (4.39), 263 (4.91), 230 (4.49). MS *m/z*: 300.0982 (M^+, Calcd for $C_{17}H_{16}O_5$: 300.0997), 285, 253, 242, 227. IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1615, 1605, 1575 (phenyl nucleus). ^{1}H-NMR (in acetone-d_6): Table III.

2,7-Diacetoxy-3,4,8-trimethoxyphenanthrene (2a)——**2** was acetylated by the method described above to yield **2a**, colorless needles, mp 159—161 °C. MS *m/z*: 384 (M^+), 342 ($M^+ - CH_2CO$), 300 ($M^+ - 2CH_2CO$, base peak), 285, 253, 242, 227. IR ν_{max}^{KBr} cm^{-1}: 1760, 1615, 1600, 1570. ^{1}H-NMR (in acetone-d_6): Table III.

2,7-Dihydroxy-3,4-dimethoxy-9,10-dihydrophenanthrene (3)——Colorless columns, mp 132—134 °C ($CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 281 (4.27), 216 (4.36). MS *m/z*: 272.1072 (M^+, Calcd for $C_{16}H_{16}O_4$: 272.1048), 257, 225, 214. IR ν_{max}^{KBr} cm^{-1}: 3370 (OH), 1615, 1590 (phenyl nucleus). ^{1}H-NMR (in acetone-d_6) δ: 2.63 (4H, s, H_2-9, 10), 3.71, 3.85 (each 3H, s, OMe), 6.56 (1H, s, H-1), 6.68—6.79 (2H, m, H-6, 8), 8.08 (1H, d, J=9.2 Hz, H-5), 7.56, 8.21 (each 1H, br s, OH). ^{13}C-NMR (in acetone-d_6) δ: 61.0, 61.2 (each q, OMe), 118.8 (d, C-1), 114.1* (d, C-8), 115.3* (d, C-6), 120.6 (s, C-12), 125.4 (s, C-11), 129.1 (d, C-13), 129.1 (s, C-5), 135.1 (s, C-14), 140.1 (s, C-3), 149.6 (s, C-2), 152.1 (s, C-4), 156.6 (s, C-7). * *Assignments may be interchanged.*

2,7-Diacetoxy-3,4-dimethoxy-9,10-dihydrophenanthrene (3a)——**3** was acetylated by the method described above to yield **3a**, colorless columns, mp 128—130 °C. MS *m/z*: 356 (M^+), 314 ($M^+ - CH_2CO$, base peak), 272 ($M^+ - 2CH_2CO$), 257, 225. IR ν_{max}^{KBr} cm^{-1}: 1765, 1610, 1590. ^{1}H-NMR (in $CDCl_3$) δ: 2.31, 2.34 (each 3H, s, OAc), 2.75 (4H, s, H_2-9, 10), 3.74, 3.90 (each 3H, s, OMe), 6.73 (1H, s, H-1), 6.96—7.02 (2H, m, H-6, 8), 8.32 (1H, d, J=9.2 Hz, H-5).

Dehydrogenation of 3a——DDQ (8.5 mg) was added to **3a** (5 mg) in dry benzene (5 ml) and the mixture was refluxed for 12 h. The benzene solution was washed with saturated $NaHCO_3$ solution, dried and evaporated under reduced pressure, yielding the phenanthrene (3.5 mg), which was identical with **1a** in terms of the ^{1}H-NMR spectrum.

3,4'-Dihydroxy-5,5'-dimethoxydihydrostilbene (4)——Colorless oil. UV λ_{max}^{MeOH} nm (log ε): 281 (3.61), 226 (4.07). MS *m/z*: 274.1188 (M^+, Calcd for $C_{16}H_{18}O_4$: 274.1204), 137 (base peak), 85, 83. IR ν_{max}^{neat} cm^{-1}: 3400 (OH), 1610, 1595 (phenyl nucleus). ^{1}H-NMR (in $CDCl_3$) δ: 2.80 (4H, s, bridge methylene), 3.74, 3.83 (each 3H, s, OMe), 5.18, 5.52 (each 1H, br s, OH), 6.24 (2H, d, J=1.8 Hz, H-2, 6), 6.30 (1H, d, J=1.8 Hz, H-4), 6.61 (1H, d, J=1.8 Hz, H-6'), 6.65 (1H, d, J=7.3 Hz, H-3'), 6.83 (1H, dd, J=7.3, 1.8 Hz, H-2'). ^{13}C-NMR (in $CDCl_3$) δ: 37.2, 38.2 (each t, bridge methylene), 55.2, 56.0 (each q, OMe), 99.1 (d, C-4), 106.7 (d, C-6), 108.3 (d, C-2), 111.3 (d, C-6'), 114.3 (d, C-3'), 121.0 (d, C-2'), 133.7 (s, C-1'), 143.8 (s, C-1), 144.5* (s, C-4'), 146.3* (s, C-5'), 156.9 (s, C-3), 160.8 (s, C-5). * Assignments may be interchanged.

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Thanks are also due to Prof. T. Namba and Associate Prof. M. Hattori, Research Institute for Wakan-Yaku of this University for ^{1}H- and ^{13}C-NMR spectral measurements.

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<添付論文 4>

Anti-inflammatory Constituents of Topically Applied Crude Drugs. IV.[1] Constituents and Anti-inflammatory Effect of Paraguayan Crude Drug "Alhucema" (*Lavandula latifolia* VILL.)[2]

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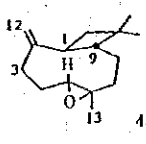
The anti-inflammatory active fraction of the Paraguayan crude drug, "Alhucema," *Lavandula latifolia* VILL. afforded four compounds: coumarin (1), 7-methoxycoumarin (2), *trans*-phytol (3) and caryophyllene oxide (4). 1 showed a weakly inhibitory effect on carrageenin-induced paw edema in rats on topical application and 4 showed an inhibitory effect on histamine-induced contraction in guinea pig ileum.

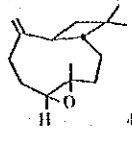
Keywords *Lavandula latifolia*; coumarin; 7-methoxycoumarin; *trans*-phytol; caryophyllene oxide; anti-inflammatory effect; carrageenin edema; histamine-induced contraction

In the course of a search for biologically active substances from Paraguayan medicinal plants, "Alhucema," the aerial portion of *Lavandula latifolia* VILL. (Labiatae) was found to have inhibitory activity when topically applied to carrageenin-induced paw edema (CPE) in rats. Alhucema is a folk medicine used for the treatment of bronchitis, asthma and rheumatism[3] and has also been topically applied to a swellings in Paraguay. It is of interest that a 70% ethanolic extract of Alhucema showed an inhibitory effect on CPE by our experimental method.[4] Studies on constituents of the essential oil of this plant have been made,[5] but no report has been found on the biological activities including its anti-inflammatory benefit.

In this paper, we report the isolation of chemical constituents and their anti-inflammatory effect. The 70% ethanolic extract was partitioned between chloroform and water to give an active chloroform soluble fraction which showed potent inhibition to CPE, and was chromatographed on a silica gel column by elution with a chloroform–methanol mixture to give an effective fraction (fr. 1) (1% methanol eluate) (Table I). Fraction 1 was further separated and purified by centrifuge liquid chromatography (CLC) and preparative thin layer chromatography (TLC) (each silica gel) to yield four compounds, 1—4. Compounds 1 and 2 were identified as coumarin and 7-methoxycoumarin, respectively, by direct comparison with authentic samples. The spectral data of compounds 3 and 4 matched those published for *trans*-phytol[6] and caryophyllene oxide.[7] 4 has four possible conformers due to the conformation of epoxide (4a, b and two *cis*-oxides). It was reported that the chemical shifts of 13-methyl group and exomethylene group were different by each isomer in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum.[8] We concluded the structure of 4 to be 4a by comparison with published data. We tested for the anti-inflammatory activity of the isolated compounds (1—4) in the CPE test using topical application; coumarin (1) showed a weakly positive effect (Table I), but the other compounds provided no reliable data owing to their limited sample availability. We previously reported that inhibition of histamine-induced contraction in guinea pig ileum (HCl) was responsible for the effect in the CPE test which could be achieved with a smaller amount of samples than in the CPE test.[1,9] The inhibitory effect of compounds 2—4 on HCl was therefore examined along with compound 1 and fr. 1 to deduce their anti-inflammatory activity; 4 showed the strongest effect ($IC_{50} = 1.0 \times 10^{-4}$ M) among them (Table II); this compound might be expected to show an inhibitory effect on CPE and further separation is in progress. In conclusion, the anti-inflammatory effect of Alhucema when topically applied is seen when combined with coumarins (1 and 2) and caryophyllene oxide (4).



4a



4b

Experimental

Melting points were determined on a Yanagimoto micromelting point

TABLE I. Inhibitory Effect on Carrageenin-Induced Paw Edema in Rats[a] by Topical Application

	Dose (mg/site × 4)	Inhibition of swelling (%)			
		1 h	2 h	3 h	4 h[b]
70% EtOH ext.	5	—	18.1	30.1[c]	18.5
$CHCl_3$ soluble	5	31.8	10.0	32.5[d]	24.3[c]
Fr. 1	5	44.8[d]	40.0[d]	45.5[d]	50.1[d]
Coumarin (1)	5	20.5	11.5	22.6[c]	19.0

a) $n = 4$ or 5. *b*) Time after carrageenin injection. —: no effect (less than 10%). *c*) $p < 0.05$. *d*) $p < 0.01$.

TABLE II. Inhibitory Effect on Histamine[a]-Induced Contraction in Guinea Pig Ileum

	IC_{50}	
	(g/ml)	(M)
Fr. 1	7.0×10^{-5}	
Coumarin (1)	$> 10^{-4}$	$> 10^{-4}$
7-Methoxycoumarin (2)	7.9×10^{-5}	4.5×10^{-4}
trans-Phytol (3)	$> 10^{-4}$	$> 10^{-4}$
Caryophyllene oxide (4)	2.4×10^{-5}	10^{-4}
Diphenhydramine·HCl	3.2×10^{-8}	1.1×10^{-7}

a) At 10^{-7} g/ml.

apparatus and were uncorrected. Spectral data were obtained as follows: infrared (IR) spectra with a Hitachi 260-0611 spectrophotometer; ultraviolet (UV) spectra with a Hitachi 270S spectrophotometer; mass spectra (MS) with a JEOL JMS-D 200 spectrometer; ^{1}H-NMR spectra with a JEOL FX 90Q (90 MHz) spectrometer; ^{13}C-NMR spectra with a JEOL FX 90Q (22.5 MHz) spectrometer. Chemical shifts were given in δ (ppm) values referred to internal tetramethylsilane (TMS). The following abbreviations were used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet.

Plant Materials Aerial parts of *Lavandula latifolia* were collected at Caacupe, Paraguay in November, 1985 and 1986 (voucher specimen deposited).

Bioassay Anti-inflammatory activity by topical application toward CPE and inhibitory effect on HCl were assessed as described in a previous report.[4]

Extraction and Separation The aerial parts of *Lavandula latifolia* (0.65 kg) were extracted with hot 70% EtOH and concentrated *in vacuo* to give 70% EtOH extract (164 g). The 70% EtOH extract was partitioned between H_2O and $CHCl_3$ to give $CHCl_3$ soluble fraction (22.6 g), H_2O soluble fraction (120.2 g) and precipitate (6.0 g). $CHCl_3$ soluble fraction was applied to column chromatography on silica gel with the $CHCl_3$–MeOH gradient system as eluent to afford fr. 1 (0.84 g, from 99 : 1), fr. 2 (5.39 g, from 97 : 3), fr. 3 (6.44 g, from 9 : 1) and fr. 4 (8.07 g, from 1 : 1) on monitoring with TLC. Fraction 1 was further separated and purified by CLC, column chromatography and preparative TLC to yield compounds **1** (0.22 g), **2** (0.027 g), **3** (0.026 g) and **4** (0.006 g). **1** (mp 70 °C) and **2** (mp 120—125 °C) were identified as coumarin and 7-methoxycoumarin by direct comparison with authentic samples.

***trans*-Phytol** Colorless oil. EI-MS *m/z*: 296 (M^+), 278 ($M^+ - H_2O$), 263, 236, 196. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600—3200, 2930, 2840, 1460. ^{1}H-NMR ($CDCl_3$) δ: 0.90 (12H, d, J = 6.0), 1.64 (3H, s, 3a-Me), 1.95 (2H, m), 4.05 (2H, d, J = 6.4, H-1), 5.33 (1H, t, J = 6.4, H-2). ^{13}C-NMR ($CDCl_3$) δ: 140.26 (s, C-3), 123.08 (d, C-2), 59.39 (t, C-1), 39.87 (t, C-4), 39.38 (t, C-14), 37.43 (t, C-10), 37.36 (t, C-8), 37.28 (t, C-12), 36.66 (t, C-6), 32.79 (t, C-11), 32.70 (t, C-7), 27.99 (d, C-15), 25.14 (t, C-5), 24.82 (t, C-13), 24.48 (t, C-9), 22.72, 22.64 (each q, C-15a, 16), 19.73 (q, C-7a, 11a), 16.16 (q, C-3a).

Caryophyllene Oxide (4) Colorless oil, $[\alpha]_D^{23}$ −64.2° (c = 0.6, $CHCl_3$). HR-MS Calcd for $C_{15}H_{24}O$: 220.1826. Found: 220.1779. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3075, 1630, 1450. ^{1}H-NMR ($CDCl_3$) δ: 0.99, 1.01 (each 3H, s, 14,15-Me), 1.20 (3H, s, 13-Me), 4.86 (1H, d, J = 1.5, =CH_2), 4.98 (1H, d, J = 1.5, =CH_2). ^{13}C-NMR ($CDCl_3$) δ: 151.8 (s, C-2), 112.7 (t, C-12), 63.7 (d, C-5), 59.8 (s, C-6), 50.9 (d, C-9), 48.7 (d, C-1), 39.8[a] (t, C-3), 39.2[a] (t, C-7), 34.0 (s, C-11), 30.2 (t, C-4), 29.9[b] (t, C-10), 29.9[b] (q, C-13), 27.3 (t, C-8), 21.6 (q, C-14), 17.0 (q, C-15). *a*) The assignments may be interchanged. *b*) Confirmed by INEPT experiment.

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<添付論文5>

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CHEMICAL AND PHARMACEUTICAL STUDIES ON MEDICINAL PLANTS IN PARAGUAY.[1] GERANIIN, AN ANGIOTENSIN-CONVERTING ENZYME INHIBITOR FROM "PARAPARAI MI," *PHYLLANTHUS NIRURI*

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"Paraparai mi," *Phyllanthus niruri* L. (Euphorbiaceae), has traditionally been used for the treatment of urolitic disease and as a diuretic in Paraguay. In the screening tests for biological activity of about 60 Paraguayan medicinal plants, however, the 70% EtOH extract of "paraparai mi" was found to have inhibitory activity against angiotensin-converting enzyme (ACE). In continuation of our studies, the *n*-BuOH extract of the medicinal plant was found to have higher activity than the EtOH extract. ACE is a dipeptidyl carboxypeptidase that plays an important role in blood pressure regulation by catalyzing an important reaction. Several inhibitors of ACE from plant sources have been reported. Previously, Inokuchi and coworkers investigated ACE inhibitors extracted from Chinese crude drugs (2,3) and reported the inhibitors from the seeds of *Areca catechu* (4). We also reported inhibitory activity of crude extracts from natural products on ACE (5). Recently, Uchida *et al.* isolated some condensed tannins from "rhei rhizoma" as ACE inhibitors (6), and Kameda *et al.* reported (+)-catechin as the inhibitor from *Quercus stenophylla* (7). *P. niruri* has already afforded alkaloids (8), lignans (9–12), flavonoids (13–16), lup-20(29)-en-3β-ol (17), phthalic acid ester (18), fatty acid (19), and vitamin C (20). In pharmacological studies of the plant, diuretic action (21) and inhibition of induced liver injury (22) have been reported.

In this paper we report the substances that inhibit ACE activity from this medicinal plant. The *n*-BuOH extract, which was the active extract, was applied to a column of Sephadex LH-20, which was eluted with H_2O followed by 50% MeOH, MeOH, and Me_2CO. Based on the results of the activities of the extracts and eluates, it seemed that the active component in the EtOH extract was extracted with *n*-BuOH and then concentrated in the 50% MeOH eluate (Table 1). The 50% MeOH eluate was further chromatographed over a Sephadex LH-20 column and was eluted with 70% MeOH to afford three compounds. They were identified as ellagic acid, geraniin, and gallic acid by direct comparison with authentic samples. The isolated compounds and corilagin, a hydrolysis product from geraniin, were tested for inhibitory activity against ACE together with captopril. The 50% inhibitory concentration (IC_{50}) values of the test compounds are shown in Table 1. It was found that the IC_{50} for ACE activity of geraniin, the most active of the isolated compounds, was 4.0×10^{-4} mol/liter. In order to determine the type

[1]This work was a part of the international collaborative study between Japan and the Republic of Paraguay supported by the funds of Japan International Agency (JICA). For the previous paper in this series, see Arisawa *et al.* (1).

TABLE 1. Inhibitory Activity of Extracts, Elutions, and Isolated Compounds from *Phyllanthus niruri* on Angiotensin-Converting Enzyme (ACE).

Extracts	Inhibition % (100 μg/ml)	Eluates	Inhibition % (100 μg/ml)	Compounds	IC_{50} (mol/liter)
70% EtOH	34	H_2O	22	ellagic acid	5.0×10^{-3}
n-hexane	14	50% MeOH	60	geraniin	4.0×10^{-4}
precipitate	40	MeOH	40	gallic acid	7.7×10^{-3}
$CHCl_3$	27	Me_2CO	17	corilagin	3.7×10^{-3}
n-BuOH	50			captopril	5.0×10^{-9}
H_2O	30				

of inhibition, the kinetics of inhibition of ACE by geraniin were plotted according to Lineweaver and Burk. Geraniin produced a noncompetitive inhibition pattern at the concentration of IC_{50}. Ondetti *et al.* found that ACE was a zinc-containing metalloprotein (23). On the other hand, Okuda *et al.* reported that geraniin interacted with the heavy metal ions (24). The present study found that geraniin was a noncompetitive inhibitor for the enzyme. This result suggested that geraniin might interact with the zinc atom in ACE.

EXPERIMENTAL

MATERIALS.—Plant material, "paraparai mi," was purchased from local dealers in Asuncion, Paraguay. It was identified as *P. niruri*, and its voucher specimens are on deposit at both the institute of Toyama Medical and Pharmaceutical University and Asuncion University. Rabbit lung ACE was purchased from Sigma Chemical Co. and hippuryl-L-histidyl-L-leucine was from the Protein Research Foundation. All other chemicals were of analytical grade.

ACE ASSAY.—Test substances were dissolved in DMSO, and the fluorometric assay was carried out by the method of Friedland and Silverstein (25) as described previously (5). The inhibitory activities are shown in Table 1.

EXTRACTION, SEPARATION, AND ISOLATION.—Dried powder of the material (185 g) was extracted with hot 70% EtOH (1 h×3). The EtOH solution was concentrated in vacuo to give a 70% EtOH extract (40 g). The EtOH extract was partitioned between H_2O and *n*-hexane to afford *n*-hexane and aqueous layer and precipitate. The aqueous layer was successively extracted with $CHCl_3$ and *n*-BuOH to give $CHCl_3$ extract (1.5 g), *n*-BuOH extract (11 g), and H_2O extract (17 g), respectively. The *n*-BuOH extract was chromatographed on a Sephadex LH-20 column by stepwise elution with H_2O, 50% MeOH, MeOH, and Me_2CO. The 50% MeOH eluate was further chromatographed on a Sephadex LH-20 eluting with 70% MeOH to afford ellagic acid (20 mg), geraniin (430 mg), and gallic acid (5 mg). Ellagic acid and gallic acid are considered to be artifacts from geraniin.

IDENTIFICATION.—The isolated compounds were identified by direct comparison (uv, ir, ^1H and ^{13}C nmr, $[\alpha]D$) with the respective authentic samples.

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<添付論文6>

—Note—

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Chemical and Pharmaceutical Studies on Medicinal Plants in Paraguay[1] Constituents of Angiotensin Converting Enzyme Inhibitory Fraction from "Cocu," *Allophylus edulis* RADLK

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Seven phenolic compounds, bergenin (1), quercetin 3-*O*-(2″-*O*-galloyl)-glucoside (2), vitexin 2″-*O*-α-L-rhamnoside (3), isovitexin (4), vicenin 2 (5), vitexin (6) and quercetin 3-*O*-β-D-glucuronide (7) were isolated from Paraguayan drug, "Cocu," *Allophylus edulis* RADLK (Sapindaceae). The isolated compounds were tested for their inhibitory activity on angiotensin converting enzyme (ACE).

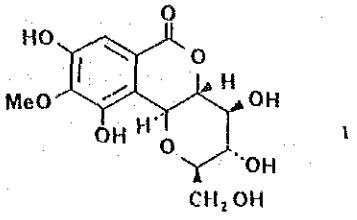
Keywords——*Allophylus edulis*; bergenin; quercetin glycoside; C-glycosyl apigenin; angiotensin coverting enzyme inhibition

The Paraguayan crude drug "Cocu" consists of the branches and leaves of *Allophylus guaraniticus* (ST. HIL JUSS et CAMB) RADLK or *A. edulis* (ST. HIL JUSS et CAMB) RADLK of the Sapindaceae family. The crude drug has been used for the treatment of liver and digestive troubles and cholecystitis. The juice of the leaves is also used for jaundice. In the screening tests for biological activities of about 60 Paraguayan medicinal plants, the 70% ethanolic extract of the crude drug derived from *Allophylus edulis* RADLK was found to have an inhibitory activity on angiotensin converting enzyme (ACE) and β-glucuronidase and cytotoxity on KB cells. Phenylacetamide[2] and 4-β-D-glucopyranosyloxy-3-hydroxymethylbuytyronitril-2-ene[3] were isolated from another plant of the same genus, *A. cobbe*, though the chemical and pharmaceutical studies on the drug have not been reported.

In this paper, we report the isolation of six flavonoids (2–7) and a dihydroisocoumarin (1) from the ACE inhibitory fraction of the crude drug and their inhibitory activity. The *n*-buthanol extract, which was the active fraction as described in the Experimental section, was applied to a column of Sephadex LH-20, and the column was eluted with aqueous methanol to give seven phenolic compounds, 1–7, which were identified as bergenin (1), quercetin 3-*O*-(2″-*O*-galloyl)-glucoside (2), vitexin 2″-*O*-α-L-rhamnoside (3), isovitexin (4), vicenin 2 (5), vitexin (6) and quercetin 3-*O*-glucuronide (7), respectively. The isolated compounds 1–7 were tested for the inhibiory activity on ACE. Their 50% inhibitory concentration (IC_{50}) values are shown in TABLE I. Compounds 3–6 showed IC_{50} values similar to those of geraniin[1] and (+)- and (−)-catechin.[4] In order to determine the mode of the inhibition activity the kinetics of the inhibition of ACE by compounds 3–6 were plotted according to Lineweaver and Burk. The four compounds produced noncompetitive inhibition patterns at the respective IC_{50} concentrations.

Experimental

General procedures——All melting points were determined on a Yanagiomoto micro melting point apparatus and are uncorrected. UV spectra were determined by the standard procedures[5] on a Hitachi 220 S double beam spectrophotometer and IR spectra in KBr disk were taken on a Hitachi 260-10 infrared spectrophotometer with polystyrene caribration at 1601 cm^{-1}. Specific rotations were determined on JASCO D-IP-140 digital polarimeter. ^{1}H-NMR and ^{13}C-NMR spectra were taken with a JEOL JNM-GX270 (at 270 MHz) and a Varian XL-200 (at 50.3



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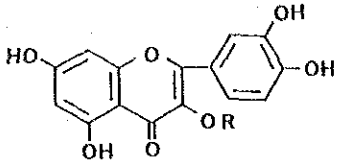
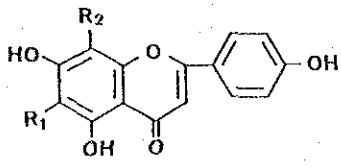


2; R= 2-O-galloylglucosyl

7; R= glucuronyl



3; R_1= H, R_2= 2-O-α-L-rhamnosylglucosyl

4; R_1= glucosyl, R_2= H

5; R_1= R_2= glucosyl

6; R_1=H, R_2= glucosyl

Chart 1.

TABLE I. Inhibitory Activity of Extracts and Isolated Compounds from *Allophylus edulis* on Angiotensin Converting Enzyme (ACE) (*in vitro*)

Extracts	Inhibition % (100 μg/ml)	Compounds	IC_{50} (mol/liter)	Compounds	IC_{50} (mol/liter)
70% EtOH	34	1	$> 1.0 \times 10^{-2}$	6	3.0×10^{-4}
n-hexane	5	2	2.7×10^{-3}	7	3.5×10^{-3}
precipitate	32	3	2.8×10^{-4}	captopril	5.0×10^{-9}
$CHCl_3$	11	4	2.8×10^{-4}	geraniin	4.0×10^{-4}
n-BuOH	53	5	2.0×10^{-4}	(+)-catechin	1.55×10^{-4}
H_2O	11			(−)-catechin	2.16×10^{-4}

MHz) spectrometers, respectively, and the chemical shifts are given in δ (ppm) with TMS as an internal standard. Mass spectra were obtained with a JEOL JMS-D-200 mass spectrometer operating at 70 eV.

Material——Plant material, "Cocu," was purchased from local dealers in Asuncion, Paraguay. It was identified as *A. edulis*, and its voucher specimens are on deposit at both the Institute of Toyama Medical & Pharmaceutical University and Asuncion University. Rabbit lung ACE was purchased from Sigma Chemical Co. and hippuryl-L-histidyl-L-leucine was from the Protein Research Foundation. All other chemicals were of analytical grade.

Extraction and separation——The dried material (1 kg) was powdered and extracted with hot 70% EtOH (1h × 3). The EtOH solution was concentrated *in vacuo* to give a 70% EtOH extract (100 g). The EtOH extract was partitioned between H_2O and *n*-hexane to give *n*-hexane (7.1 g) and aqueous (50 g) layers and precipitate (25 g). The aqueous layer and the precipitate were successively extracted with $CHCl_3$ and *n*-BuOH to give $CHCl_3$ extract (2 g), *n*-BuOH extract (11.4 g) and H_2O extract (39.3 g), respectively.

Isolation of compounds 1-7——The *n*-BuOH extract was chromatographed on a Sephadex LH-20 column by stepwise elution with H_2O, aqueous MeOH and MeOH. The H_2O eluent afforded 1 (230 mg) and the aqueous MeOH eluent afforded 2 (25 mg), 3 (30 mg), 4 (15 mg), 5 (20 mg), 6 (18 mg) and 7 (27 mg).

ACE assay——Test substances were dissolved in DMSO, and the fluorometric assay was carried out by the method of Friedland and Silverstein[6] as described previously.[1,7] The inhibitory activities are shown in TABLE I.

Identification of compounds **1**-**7**——**1**: colorless prisms, mp 138–139°C, $[\alpha]_D^{25}$ -37.3° (c = 0.1, EtOH). Compound **1** was suggested to be bergenin by its spectral data (UV, IR, MS, ^{1}H-NMR and ^{13}C-NMR), and was identified as bergenin by direct comparison with an authentic sample isolated from the bark of *Mallotus japonicus*. **2**: yellow needles, mp 211–213°C. The spectral data were identical with those reported.[8] Compound **2** was identified as quercetin 3-*O*-(2″-*O*-galloyl)-glucoside by direct comparison with an authentic sample isolated from *Polygonum lapathifolium* L. subsp. *nodosum* (PERS.) KITAN.[9] **3**: fine yellow needles, mp 208–210°C. The ^{1}H-NMR data of its acetate were identical with the reported values[10] and the ^{13}C-NMR spectrum of **3** was also identical with the reported spectrum of vitexin 2″-*O*-α-L-rhamnoside.[11] **4**: fine yellow needles, mp 223–224°C. Compound **4** was identified as isovitexin by direct comparison (mixed mp, UV, IR, ^{1}H-NMR and ^{13}C-NMR) with an authentic sample isolated from *Scoparia dulcis* L.[12] **5**: fine pale yellow needles, mp 227–229°C. Compound **5** was identified as vicenin 2 by direct comparison (mixed mp, UV, IR, ^{1}H-NMR and ^{13}C-NMR) with an authentic sample.[12] **6**: yellow needles, mp 269–270°C. Compound **6** was identified as vitexin by direct comparison (mixed mp, UV, IR, ^{1}H-NMR and ^{13}C-NMR) with an authentic sample.[12] **7**: yellow needles, mp 206–208°C. Compound **7** was identified as quercetin 3-*O*-β-D-glucuronide by direct comparison (mixed mp, UV, IR, ^{1}H-NMR and ^{13}C-NMR) with an authentic sample.[9]

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<添付論文7>

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Studies on Medicinal Plants in Paraguay; Studies on "Romero"; Part 1

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"Romero" (*Rosmarinus officinalis* L., Labiatae) is one of the important folk medicines in Paraguay. The leaves of this plant have been used as stimulant, stomachic and hepatic agents (1).

In the course of a search for biologically active substances from Paraguayan medicinal plants, the *n*-hexane layer and the precipitate from the aqueous layer of 70 % ethanol extract of the dried leaves of "Romero" were found to exhibit *marked inhibitory activities against* urease (95 %) and KB cells (87 %) at 50 μg/ml, respectively. The inhibitor of urease might be expected to act as a therapeutic agent for urolithiasis (2). The KB assay is a well known method to find the anti-cancer agent in natural resouces (3).

Thus, the *n*-hexane extract (11 g) was chromatographed on a silica gel column by elution with benzene-acetone mixture followed by Sephadex LH-20 column eluting with methanol to afford the compounds **1** (8 mg), **2** (13 mg), and **3** (130 mg). The chloroform-soluble part (7 g) of the precipitate was also chromatographed on a silica gel column by elution with chloroform to afford **3** (210 mg), **4** (7 mg), and **5** (110 mg). The compounds **2**–**4** were identified as rosmanol (**2**), carnosol (**3**), and genkwanin (**4**), respectively, by direct comparison with authentic samples which had been previously reported from the same plant (4–6). The compound **5** was identified as betulinic acid by comparison of spectral data of **5** and its methyl ester with those of published values (7, 8). The isolation of betulinic acid from this plant is described for the first time.

Compound **1**, colorless needles, m. p. 218–223° C, $C_{22}H_{30}O_5$ was identified as 7-ethoxyrosmanol by comparison of the spectral data of **1** with those of rosmanol (**2**). The compound **1** was obtained by heating rosmanol (**2**) with aqueous ethanol in the presence of silica gel which is suggesting **1** to be an artifact.

Then, the isolated five compounds **1**–**5** were subjected to an examination of inhibitory activity against urease (9) and KB cells (10). As indicated in Table I, rosmanol (**2**) showed inhibitory activity against urease, while no compounds showed potent cytotoxicity.

Further separation and purification of active substances are currently being performed.

Table I. Inhibitory activities of the isolated compounds against urease and KB cells in culture

Compounds	IC_{50}	
	Urease (mol/ml)	KB cells (μg/ml)
7-Ethoxyrosmanol (1)	$>10^{-3}$	8.4
Rosmanol (2)	4.3×10^{-5}	23.5
Carnosol (3)	$>10^{-3}$	14.7
Genkwanin (4)	$>10^{-3}$	>100
Betulinic acid (5)	$>10^{-3}$	8.4

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<添付論文 8>

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CHEMICAL AND PHARMACEUTICAL STUDIES ON MEDICINAL PLANTS IN PARAGUAY: STUDIES ON "ROMERO," PART 2

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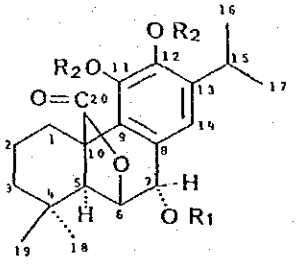
"Romero" (*Rosmarinus officinalis* L., Labiatae) is a folk medicine in Paraguay (*1*). Earlier investigation of the constituents of this plant resulted in the isolation of rosmarinic acid (2). Afterwards, this plant afforded alkaloids (3-5), flavonoids (6,7), terpenoids (8,9), diterpene quinones (10), terpenic acids (11), carnosol (12), carnosic acid (13), and phenolic acid (14). Recently, several antioxidative constituents were isolated from the same plant (15-21). Previously, we reported biological studies for this medicine, including inhibitory activities against urease and KB cells (22).

In a continuing study of "romero," a new compound, 7-methoxyrosmanol [**1**], was isolated together with two known compounds, betulin and cirsimaritin. We wish to report the structural elucidation of the new compound.

The $CHCl_3$ extract was subjected to chromatographic separation which resulted in the isolation of the three compounds. Compound **1** was a pale brown powder, $C_{21}H_{28}O_5$, whose ir spectrum was similar to those of rosmanol [**2**] and 7-ethoxyrosmanol [**3**]. The ^{1}H-nmr spectrum of **1** was also similar to that of **2**, except for the appearance of the signals of the methoxy protons at δ 3.66 (3H) ppm (Table 1). Acetylation of **1** gave a diacetate [**1a**] that had a molecular ion at *m/z* 444 in the ms spectrum. The ^{13}C-nmr spectrum of **1** showed 21 carbon signals including a carbonyl carbon, 6 aromatic carbons, and 3 carbons attached to oxygen. A carbon signal of C-7 was lower than that of **2** by 9.3 ppm



	R_1	R_2
1	Me	H
1a	Me	Ac
2	H	H
3	Et	H

and 2 carbon signals of C-6 and C-8 were higher than those of 4.1 and 2.0 ppm, respectively (Table 2). From these spectral data, the structure of **1** was determined to be 7-methoxyrosmanol [**1**]. The remaining two compounds were identified as betulin and cirsimaritin by comparison with authentic samples. This is the first time these compounds have been isolated from this plant.

EXPERIMENTAL

PLANT MATERIAL.—Aerial parts of *R. officinalis* were collected in Cacupe, the suburbs of Asuncion, Paraguay, in July 1985. The voucher specimens are on deposit both in the institute of Toyama Medical & Pharmaceutical University and at Asuncion University.

EXTRACTION AND SEPARATION.—The dried "romero" (1.9 kg) was extracted three times with hot 70% EtOH. The EtOH extract (130 g) was partitioned between *n*-hexane and H_2O to afford *n*-hexane layer, H_2O layer, and precipitate. The H_2O layer was extracted with $CHCl_3$. The $CHCl_3$ extract (14 g) was chromatographed on a Si gel column with elution by $CHCl_3$-Me_2CO (200:1), followed by a second Si gel column with

TABLE 1. ^{1}H-nmr Spectral Data of Rosmanols (δ ppm, J=Hz)

Proton No.	Compounds			
	1[a]	**1a**[a]	**2**[b,c]	**3**[b]
19-H	0.93 (3H, s)	0.93 (3H, s)	0.90 (3H, s)	0.91 (3H, s)
18-H	1.01 (3H, s)	1.01 (3H, s)	1.02 (3H, s)	1.02 (3H, s)
16-H	1.22 (d) (J=6.8)	1.18 (3H, d) (J=6.8)	1.17 (3H, d)	1.17 (3H, d)
17-H	1.22 (d) (J=6.8)	1.20 (3H, d) (J=6.8)	1.18 (3H, d)	1.20 (3H, d)
1α-H	1.98 (1H, ddd) (J=13.7, 13.7, 5.5)	1.78 (1H, m)	1.98 (1H, dddd)	1.96 (1H, m)
5-H	2.24 (1H, s)	2.23 (1H, s)	2.29 (1H, s)	2.22 (1H, s)
15-H	3.06 (1H, sept) (J=6.8)	2.89 (1H, sept) (J=6.8)	3.27 (1H, sept)	3.27 (1H, m)
1β-H	3.16 (1H, m)	2.83 (1H, m)	3.29 (1H, m)	3.30 (1H, m)
6α-H	4.24 (1H, d) (J=3.2)	4.32 (1H, d) (J=2.9)	4.52 (1H, d)	4.35 (1H, d)
7-H	4.71 (1H, d) (J=3.2)	4.70 (1H, d) (J=2.9)	4.64 (1H, d)	4.75 (1H, d)
14-H	6.79 (1H, s)	7.23 (1H, s)	6.89 (1H, s)	6.84 (1H, s)
OAc		2.28 (3H, s) 2.30 (3H, s)		

[a]In $CDCl_3$.
[b]In acetone-d_6.
[c]See Inatani *et al.* (16).

TABLE 2. ^{13}C-nmr Spectral Data of Rosmanols (δ ppm)

Carbon No.	**1**		**2**[a]	**3**
	in $CDCl_3$	in acetone-d_6	in acetone-d_6	in acetone-d_6
1	27.2, t	28.1, t	28.4, t	28.3, t
2	19.0, t	19.8, t	19.9, t	19.8, t
3	38.0, t	38.9, t	39.1, t	38.9, t
4	31.5, s	31.9, s	32.0, s	32.0, s
5	50.8, d	51.3, d	51.0, d	51.5, d
6	74.6, d	74.7, d	78.8, d	75.4, d
7	77.5, d	78.4, d	69.1, d	76.8, d
8	126.4, s	128.0, s	130.4, s	128.4, s
9	124.2, s	124.8, s	124.9, s	124.1, s
10	47.0, s	47.5, s	47.7, s	47.6, s
11	142.4, s	144.5, s	144.7, s	144.6, s
12	142.1, s	142.6, s	142.5, s	142.6, s
13	135.1, s	136.2, s	136.5, s	136.3, s
14	120.5, d	120.6, d	120.2, d	120.6, d
15	27.2, d	27.3, d	27.5, d	27.4, d
16	22.3, q	22.8, q	22.9, q	22.9, q
17	22.5, q	23.1, q	23.1, q	23.1, q
18	31.4, q	31.9, q	31.8, q	31.8, q
19	22.1, q	22.3, q	22.4, q	22.3, q
20	179.0, s	178.5, s	178.3, s	178.4, s
OCH_3	58.2, q	58.3, q		
OCH_2-				66.4, t
OCH_2CH_3				16.2, q

[a]See Inatani *et al.* (16).

elution by C_6H_6-Me_2CO (9:1) to afford crude **1** (34 mg), betulin (23 mg), and cirsimaritin (30 mg). The crude compounds were purified with plc.

CHARACTERIZATION OF 7-METHOXYROSMANOL [**1**].—Pale brown powder; $[\alpha]^{23}D$ −99.2° (*c*=0.5, EtOH); positive to $FeCl_3$; uv λ max (EtOH) (log ε) 292 (3.24), 230 (4.00), 213 (4.27) nm; ir ν max ($CHCl_3$) 3500, 2975, 1740, 1440, 1360, 1215, 1090, 1040 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; ms *m/z* 360 (M^+), 316, 314, 284, 245, 215, 149; *Anal.* calcd for $C_{21}H_{28}O_5$: 360.1935. Found (ms): 360.1941.

ACETYLATION OF **1**.—A mixture of **1** (5 mg), Ac_2O (0.1 ml), and pyridine (0.1 ml) was allowed to stand at room temperature overnight. The reaction mixture was worked up as usual to give a diacetate [**1a**] (4.5 mg). Negative to $FeCl_3$; ^1H nmr see Table 1; ms *m/z* 444 (M^+), 402, 388, 361, 360, 346, 342, 316, 314, 258, 245, 215, 43; *Anal.* calcd for $C_{25}H_{32}O_7$: 444.2146. Found (ms): 444.2132.

IDENTIFICATION OF BETULIN.—Colorless needles, mp 248-250° (MeOH); $[\alpha]^{23}D$ +11.63° (*c*=0.5, $CHCl_3$). It was identified by comparison with published values (ir, ^1H and ^{13}C nmr, ms, and $[\alpha]D$) for betulin (23-26).

IDENTIFICATION OF CIRSIMARITIN.—Yellow needles, mp 264-268° (MeOH); uv λ max (MeOH) 331, 273 nm. It was identified by direct comparison with an authentic sample isolated from *Cirsium maritium* Makino (Compositae) (27).

ACKNOWLEDGMENTS

This work was a part of the international collaborative study between Japan and the Republic of Paraguay supported by the funds of Japan International Agency (JICA). The authors thank Mr. M. Morikoshi, Analytical Center of Toyama Medical & Pharmaceutical University, for measurement of mass spectra.

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<添付論文9>

Communications to the Editor

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STRUCTURE OF SCOPARIC ACID A, A NEW LABDANE-TYPE DITERPENOID FROM A PARAGUAYAN CRUDE DRUG "TYPYCHÁ KURATŨ" (*SCOPARIA DULCIS* L.)

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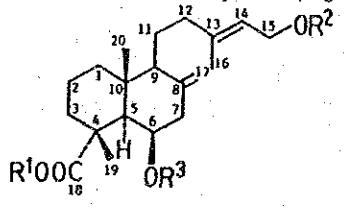
A new labdane-type diterpenoid, scoparic acid A, which has an inhibitory effect on β-glucuronidase, was isolated from *Scoparia dulcis* L. and its structure was elucidated by means of 2-D NMR spectroscopy including the INADEQUATE and ^{1}H- ^{13}C long-range COSY.

KEYWORDS ——— scoparic acid A; diterpenoid; labdane; Typychá Kuratũ; *Scoparia dulcis*; Scrophulariaceae; β-glucuronidase inhibitor; 2-D INADEQUATE; ^{1}H-^{13}C long-range COSY

"Typychá Kuratũ", whole plants of *Scoparia dulcis* L., (Scrophulariaceae), is one of the Paraguayan folk medicines often used for the treatment of stomach disease as well as for hepatosis. From the roots of this plant, 6-methoxybenzoxazolinone,[1] having a hypertensive activity, has been isolated by Taiwan workers. In the course of our search for biologically active substances from Paraguayan medicinal plants, we found that the extract of "Typychá Kuratũ" has a great inhibitory effect on β-glucuronidase. By bioassay-guided separation, we isolated a labdane-type diterpenoid, named scoparic acid A (1), which inhibited the activity of β-glucuronidase from bovine liver (IC_{50},



1 R^1=R^2=H, R^3=COC_6H_5
2 R^1=CH_3, R^2=H, R^3=COC_6H_5
3 R^1=H, R^2=$COCH_3$, R^3=COC_6H_5
4 R^1=R^2=R^3=H

$6.8X10^{-6}M$). It also mildly inhibited histamine-induced contraction of the ileum isolated from guinea pigs (IC_{50}, $3.2X10^{-5}M$). In this communication, we report the structure of 1.

The aqueous ethanol (70%) extract from dried "Typychã kuratũ" was partitioned between n-hexane and water. The precipitate obtained from the aqueous layer was chromatographed on a silica gel column using a MeOH-$CHCl_3$ stepwise gradient and a fraction eluted with MeOH-$CHCl_3$(1:9) was further purified by HPLC on a TSK-GEL Silica 60 column with the MeOH-$CHCl_3$ stepwise gradient (1:99 to 10:90) to give scoparic acid A (1) as a colorless amorphous powder.

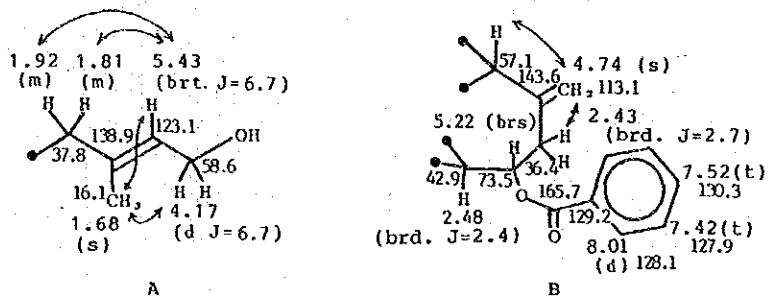


Fig.1. Partial Structures of Scoparic Acid A (δ-Values in $CDCl_3$)
(↙ long-range coupling in ^1H-^1H COSY)

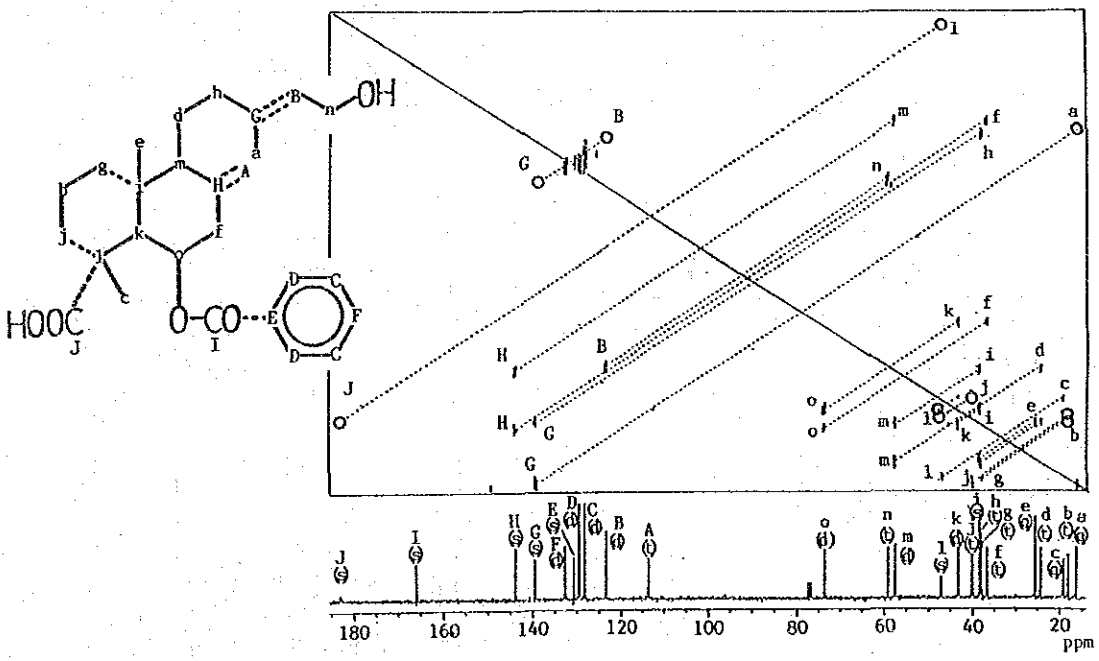


Fig.2. 2-D INADEQUATE Spectrum (Contour Map) of Scoparic Acid A (1) in $CDCl_3$

The spectrum was measured on a JEOL GX-400 spectrometer,using 170 mg of the sample (40°C, 60h run , J_{CC}=45Hz). The multiplicities of carbon signals were determined by the off-resonance and DEPT methods and indicated as s, d, t and q; sp^2 carbons are marked with A-J and sp^3 carbons with a-o in the order of increasing δ values. Open circles indicate the expected carbon signals, which were not observed in this measurement, but were observed in other experiments.

Scoparic acid A (1), $C_{27}H_{36}O_5$, $[\alpha]_D$ -38.3°($CHCl_3$), showed IR absorptions at 3400(OH), 1700(CO), 1600 and 1580 cm^{-1} (phenyl) and UV absorptions at 227, 265(sh), 270 and 277 nm. The EI-MS of 1 showed the molecular ion peak at m/z 440 and fragment ion peaks at m/z 422 (M^+-H_2O), 394 (M^+-HCOOH), 377 (M^+-H_2O-COOH), 335 ($M^+-C_6H_5CO$), 318 ($M^+-C_6H_5COOH$), 300, 173 ($C_{13}H_{17}^+$), 159 ($C_{12}H_{15}^+$), 105 ($C_6H_5CO^+$) and 77 ($C_6H_5^+$). The ^{1}H-NMR spectrum showed signals due to one vinyl methyl (δ 1.68), two tertiary-methyls (δ

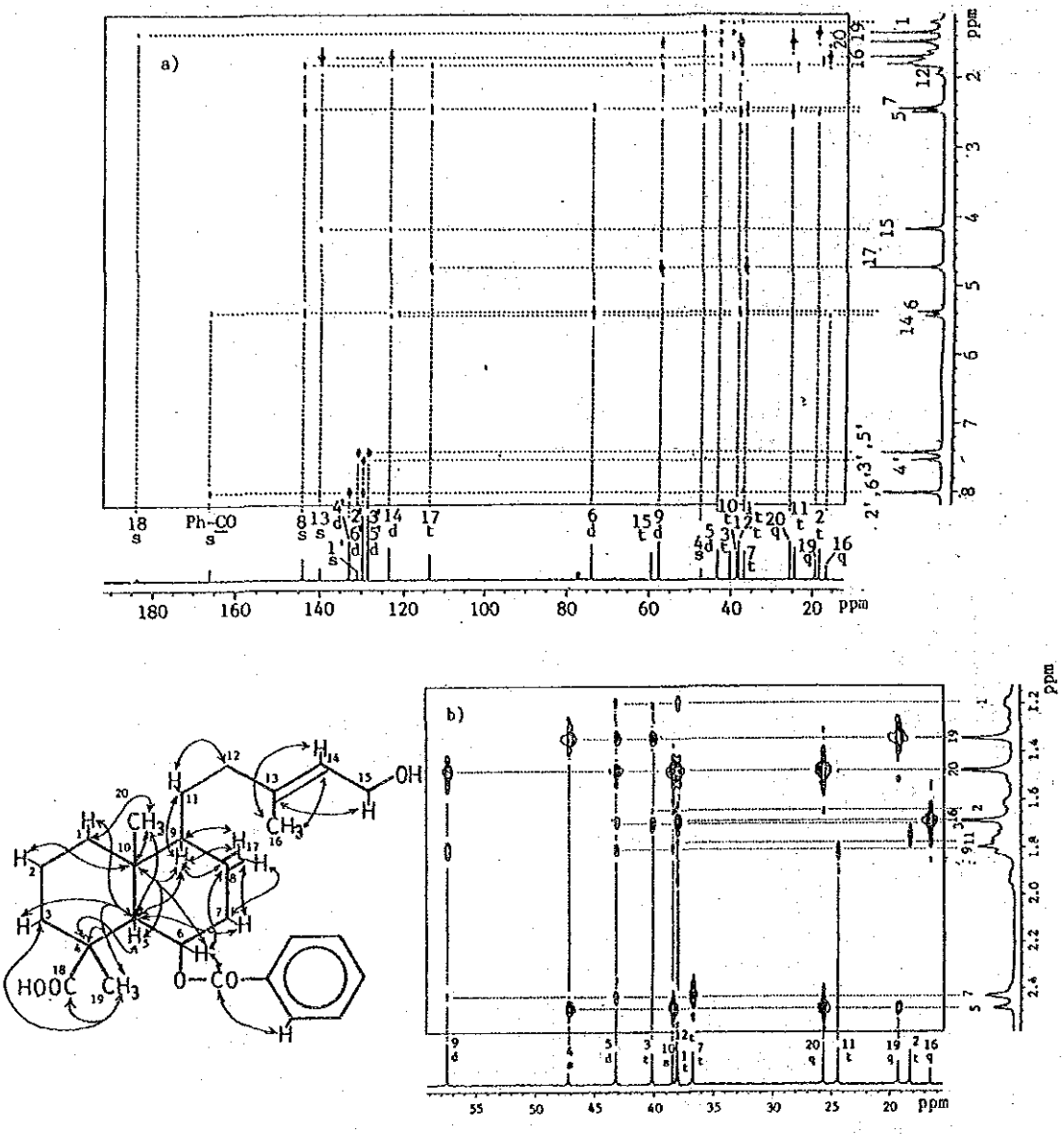


Fig.3. ^{1}H-^{13}C Long-Range Shift Correlation Spectra of Scoparic Acid A in $CDCl_3$:
a) Whole region. b) High field region
The spectra were measured using 90mg of the sample (35°C,12 h run, J_{CH}=10Hz). The ^{1}H-signals were assigned using ^{1}H-^{1}H and ^{1}H-^{13}C shift correlation spectra.

1.34 and 1.47), one carbinyl methylene (δ 4.17) and one phenyl group (δ 7.42, 2H, 7.52, 1H and 8.01, 2H), along with three olefinic protons (δ 4.74, 2H and 5.43, 1H). On treatment with diazomethane and with acetic anhydride-pyridine, 1 gave a methyl ester (2), $C_{28}H_{38}O_5$, $[\alpha]_D$ -50.2°($CHCl_3$), δ 3.69 ($COOCH_3$), and an acetate (3), $C_{29}H_{38}O_6$, δ 4.62 (d, J=7.1, $C\underline{H}_2OAc$), 2.07 ($OCOC\underline{H}_3$), respectively.

Scoparic acid A resisted hydrolysis by the usual method but was hydrolyzed by heating with 4% KOH in MeOH-DMSO *in a sealed tube yielding a debenzoylated* compound (4), $C_{20}H_{32}O_4$, δ 4.00 (H-6).

These data and detailed ^1H- and ^{13}C-NMR studies of 1 with the aid of ^1H-^1H and ^1H-^{13}C shift correlation spectroscopy led us to conclude that 1 may be a diterpene acid having the partial structure A and B (Fig. 1).

At this stage, the 2-D INADEQUATE spectra[2] of 1 were measured under various conditions to determine the carbon-carbon connectivities in the molecule. As illustrated in Fig. 2, there were correlation peaks of all the coupled ^{13}C-^{13}C pairs except those between the carbons g and i, A and H, and E and I. Therefore a labdane-type bicyclic structure was deduced for 1.

Next, we measured the ^1H-^{13}C long-range shift correlation spectrum of 1 in order to confirm the sequence of carbon atoms. As shown in Fig. 3, the methylene carbon at δ 38.0(C-1) is correlated with the methyl protons at δ 1.48 (20-H_3). In turn, the carbon atoms corresponding to the signals at δ 38.0 (C-10) and at δ 42.9 (C-5) are correlated with the protons corresponding to the signals at δ 1.48 (20-H_3), 1.61 (2-H), 1.81 (9-H), 2.50 (5-H) and 5.38 (6-H) and at δ 1.19 (1-H), 1.35 (19-H_3), 1.48 (20-H_3), 1.70 (3-H), 1.81 (9-H) and 2.44 (7-H_2), respectively. Also, some of significant ^1H-^{13}C long-range correlations are indicated by arrows in the formula in Fig. 3. Thus, the planar structure of this compound was shown to be 1.

The relative stereochemistry of scoparic acid A (1) was determined by nuclear Overhauser effect (NOE) difference spectroscopy. Irradiation of the 19- and 20-methyls enhanced the signal intensity of the 20- and 2',6'-protons and the 19-and 2',6'-protons respectively. Similarly, irradiation of the 16-methyl increased the signal intensity of the 15-metylene protons. Also, NOE's were observed between 5- and 6-protons and between the 6- and 7-protons. Thus, the relative configuration of scoparic acid A was determined as represented by the formula 1.[3]

Details of the biological activities of scoparic acid A will be reported elsewhere.

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3) The configuration of the side chain at the C-9 position was supposed to be β on the basis of stability and biogenetical view point.

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＜添付論文 10＞

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SCOPADULCIC ACID-A AND -B, NEW DITERPENOIDS WITH A NOVEL SKELETON, FROM A PARAGUAYAN CRUDE DRUG "TYPYCHÁ KURATŨ" (*SCOPARIA DULCIS* L.)

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SCOPADULCIC ACID-A AND -B, NEW DITERPENOIDS WITH A NOVEL SKELETON, FROM A PARAGUAYAN CRUDE DRUG "TYPYCHÁ KURATŨ" (SCOPARIA DULCIS L.)

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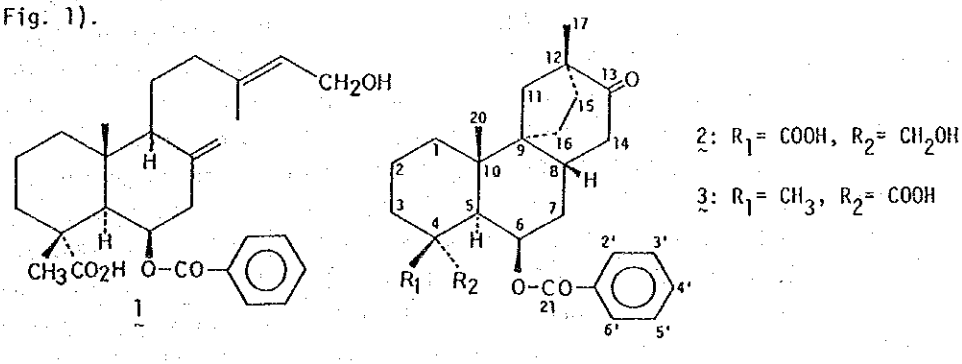
Abstract: Scopadulcic acid-A and -B, diterpenoids with a novel skeleton, have been isolated from the whole plants of Scoparia dulcis L., and their structures, including the absolute configuration, were determined based on the 2-D NMR and CD spectral data.

During our investigation on biologically active substances from Paraguayan medicinal plants, a new labdane-type diterpenoid, scoparic acid-A (1), was isolated from the 70% ethanol extract of "Typychá kuratũ" (whole plants of Scoparia dulcis L., Scrophuraliaceae).[1] From the same source, we have now isolated two novel diterpenoids, named scopadulcic acid-A (2) and -B (3). In this communication, structures of scopadulcic acid-A and -B, including their absolute stereochemistry, are described.

Scopadulcic acid-A (2), colorless prisms (MeOH), mp 172-174°, $[\alpha]_D^{27}$-5.7° (MeOH), has the molecular formula $C_{27}H_{34}O_6$ (M^+ 454.2313, calcd. 454.2354) and its UV and IR spectra showed absorption maxima at 229 (log ε:4.15), 275sh (2.98), 277 (3.02) and 280 (2.94) nm and at 3500 (OH), 1710, 1700 (CO), 1600, 1590 (phenyl) cm^{-1}, respectively. The ^1H- and ^{13}C-NMR spectra of 2 indicated the presence of two carbonyls (δ_C 178.2 and 212.8), a benzoyl (δ_H 7.43, 7.57, 7.96; δ_C 129.5, 130.8, 132.5, 133.7 and 166.8), a hydroxymethylene (δ_H 3.56 and 3.79, each d, J=10.6 Hz; δ_C 68.2) and two tert-methyl groups (δ_H 1.01 and 1.60; δ_C 20.5 and 21.6) and four quarternary sp^3 carbons (δ_C 40.0, 48.8, 53.2 and 54.2). These data coupled with the detailed analyses of the ^1H-^1H and ^1H-^{13}C COSY spectra[2] suggested that 2 has the partial structures A and B (Fig. 1).



2: R_1= COOH, R_2= CH_2OH

3: R_1= CH_3, R_2= COOH

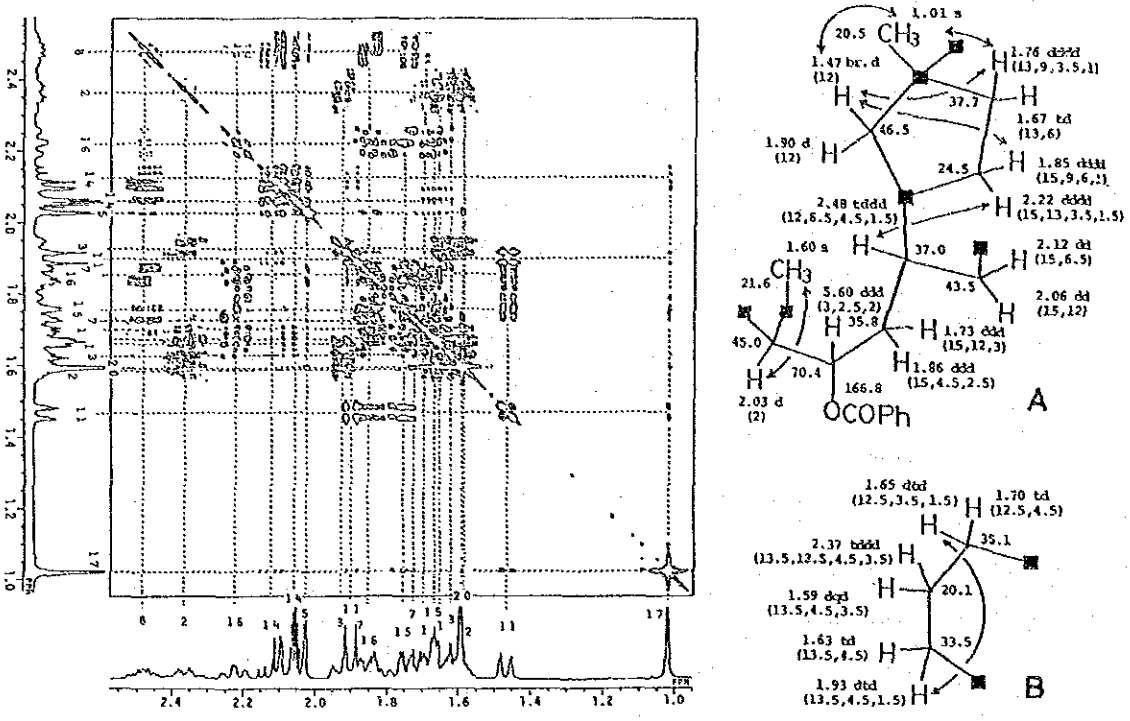


Fig. 1 Highly Resolved ^{1}H-^{1}H COSY Spectrum of Scopadulcic acid-A (2) in the High Field Region (in Acetone-d_6) and Partial Structures in 2 (⟿ : long-range coupling observed; coupling constants in parenthesis)

Then, we measured the ^{1}H-^{13}C long-range COSY spectrum of 2 in order to clarify the connectivities of the partial structures and substituent groups. As shown in Fig. 2, the carbon signal at δ45.0 (C-5) is connected with the proton signals at δ1.70 (1-H), 1.93 (3-H), 1.86 (7-H), 3.79 (18-H) and 1.60 (20-H_3) in terms of long-range correlation, while the quarternary carbon signal at δ48.8 (C-4) with the protons at δ1.93 (3-H) and 2.03 (5-H). Also, the quarternary carbon signals at δ54.2 (C-9), 40.0 (C-10) and 53.2 (C-12) are correlated with the proton signals at δ1.86 (7-H), 1.90 (11-H), 2.06 (14-H), 2.22 (16-H) and 1.60 (20-H_3), at δ1.65 (1-H), 2.03 (5-H), 5.60 (6-H) and 1.60 (20-H_3), and at δ1.90 (11-H) and 1.01 (17-H_3), respectively. Thus, the planar structure of scopadulcic acid-A was assigned to the formula 2a in Fig. 2, in which some of other significant long-range correlations observed are also shown by arrows.

The relative stereochemistry was elucidated on the basis of the coupling constants of each proton and the results of NOE experiments. Irradiation of the 20-methyl and the 8-proton caused the increase of the signal intensity of the 11-, 20-, and 2',6'-protons and the 8-, 11-, and 2', 6'-protons, respectively. Also, NOE's between the 5- and 6-protons and between the 5- and 16-protons were observed. These findings enabled us to determine the stereostructure of scopadulcic acid-A to be 2b as depicted in Fig. 3.

Scopadulcic acid-B (3), colorless prisms (MeOH), mp 228-232°, $[\alpha]_D^{27}$ -49.6° (MeOH), has the molecular formula $C_{27}H_{34}O_5$ (M^+ 438.2420, calcd. 438.2415) and it showed the UV and IR spectra very similar to those of 2. Extensive studies of the ^{1}H- and ^{13}C-NMR spectra of 3[3] compared with those of 2 indicated that both are closely related compounds with each other, but 3 has an

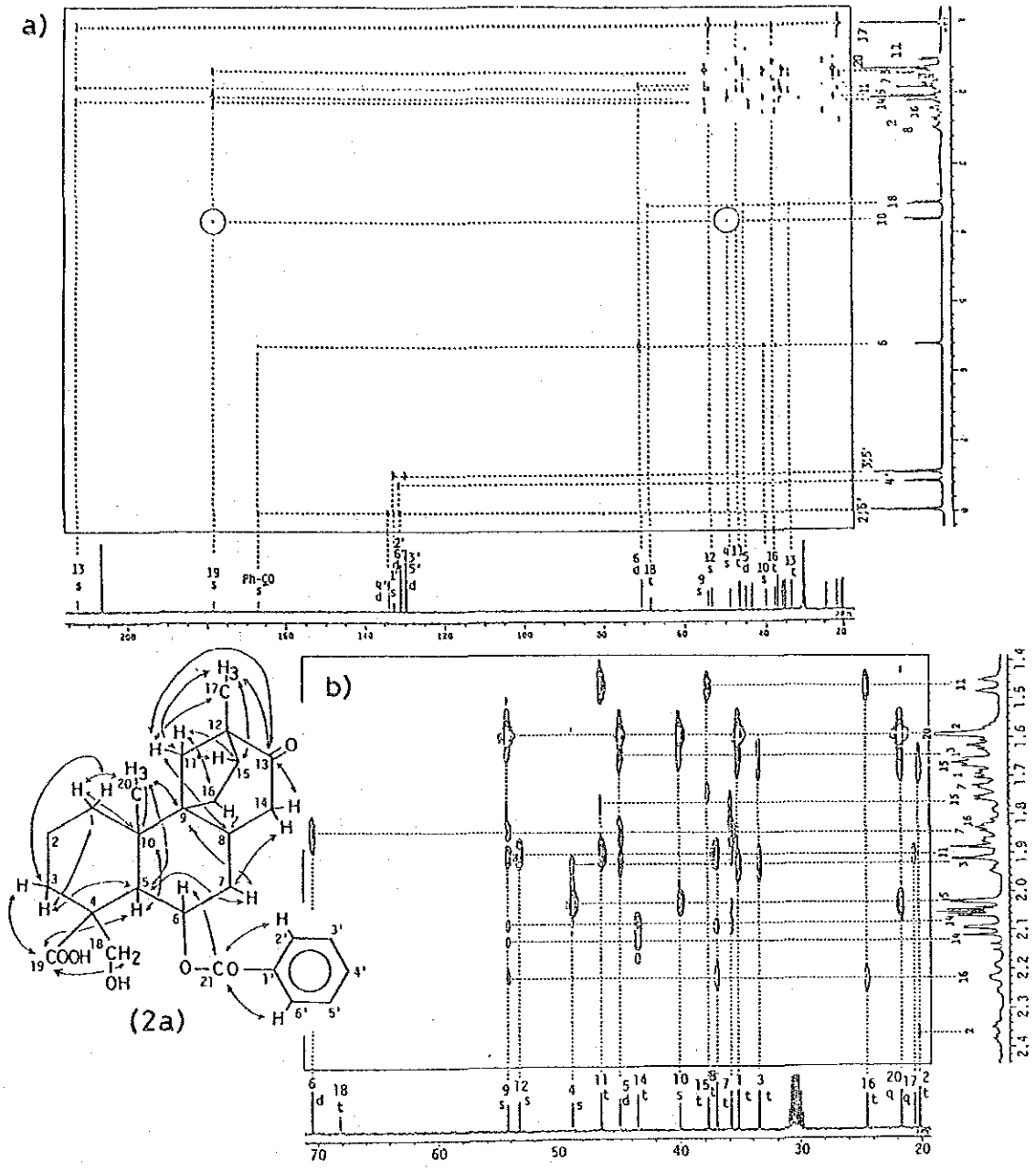


Fig. 2 ^{1}H-^{13}C Long-range COSY Spectrum of Scopadulcic acid-A (2) in Acetone-d_6
a) Whole Region, b) High Field Region (Sample: 20 mg, 20°C, J_{CH}=10 Hz, 12 hr run)
Multiplicities of carbon signals were determined by the off-resonance and DEPT methods and are indicated as s, d, t and q. Open circles indicate the correlation peaks, which are significant but weak at this threshold level.

additional tert-methyl group instead of the hydroxymethylene group in 2. Further, NOE's were observed as shown in the formula 3a (Fig. 3). Thus, the structure of scopadulcic acid-B was assigned to the formula 3.

The absolute configuration of scopadulcic acid-A and -B was determined as 2 and 3,

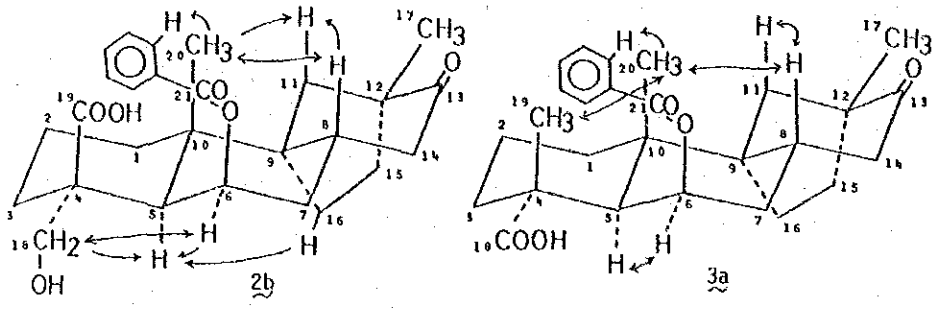


Fig. 3 NOE's Observed in Scopadulcic acid-A and -B

respectively, based on the fact that both compounds showed the positive Cotton effect due to the optically active ketone chromophore in the CD spectra (CD maximum: 2, $[\theta]_{297}^{MeOH}$ +2130; 3, $[\theta]_{297}^{CHCl_3}$ +2490). Octant projection of the structures (2b and 3a) reasonably supports these assignments.

It should be noted that scopadulcic acid-A (2) and -B (3) represent a new class of diterpenoids and particularly, they are of interest from a biogenetic view-point.[4] The biological activities of these compounds are now under examination.

Aknowledgements: This work was supported in part by a grant from Japan International Cooperation Agency and by a Grant-in-Aid for Scientific Research to T.K. (No. 61470147) from the Ministry of Education, Science and Culture of Japan.

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^{13}C-NMR of 3 ($CDCl_3$,δ): 213.6 (13-C), 184.2 (18-C), 166.1 (21-C), 133.4 (4'-C), 130.5 (1'-C), 129.6 (2',6'-C), 128.5 (3',5'-C), 72.9 (6-C), 53.1 (9-C), 52.3 (15-C), 47.2 (4-C), 45.1 (11-C), 44.6 (5-C), 42.5 (14-C), 39.7 (3-C), 38.8 (10-C), 36.6 (16-C), 36.0 (8-C), 35.1 (7-C), 34.0 (1-C), 23.7 (16-C), 21.6 (20-C), 19.7 (17-C), 19.3 (19-C), 18.0 (2-C).

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<添付論文11>

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THE CRYSTAL STRUCTURE OF SCOPADULCIC ACID A FROM PARAGUAYAN CRUDE DRUG "TYPYCHÁ KURATÛ" (*SCOPARIA DULCIS*)

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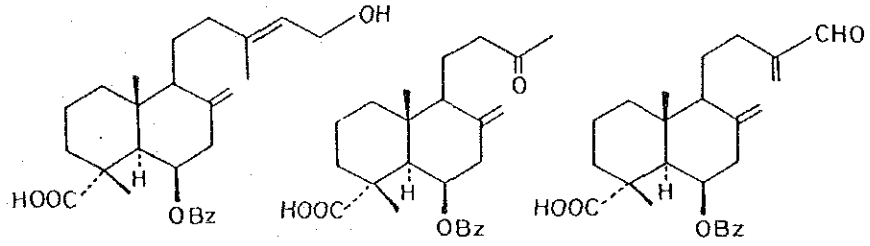
In our search for biologically active substances in Paraguayan medicinal plants, we have isolated five cytotoxic diterpenoids named scoparic acids A [**1**], B [**2**], and C [**3**], and scopadulcic acids A [**4**] and B [**5**] from whole plants of *Scoparia dulcis* L. (Scrophulariaceae). They were characterized as new diterpenoids on the basis of spectral data including 2D-nmr spectra (1–3). Among them, scopadulcic acids A [**4**] and B [**5**] were found to have a novel skeleton. Scopadulcic acid A [**4**] was obtained as colorless prisms, and its structure has now been confirmed by single crystal X-ray analysis. The crystal structure of scopadulcic acid A is illustrated in Figure 1.

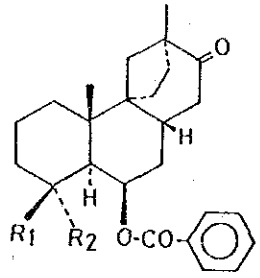
EXPERIMENTAL

PLANT MATERIAL.—Whole plants of *S. dulcis* were collected near Asuncion, Paraguay, in April 1986. The voucher specimens are deposited in the Herbal Garden of the Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, and Sección Botanica, Facultad de Ciencias Químicas, Universidad Nacional de Asunción.

EXTRACTION AND ISOLATION OF SCOPADULCIC ACID A.—The whole parts of air-dried *S. dulcis* were ground to a fine powder and



1 Bz=COC_6H_5 **2** Bz=COC_6H_5 **3** Bz=COC_6H_5



4 R_1=COOH, R_2=CH_2OH
5 R_1=Me, R_2=COOH

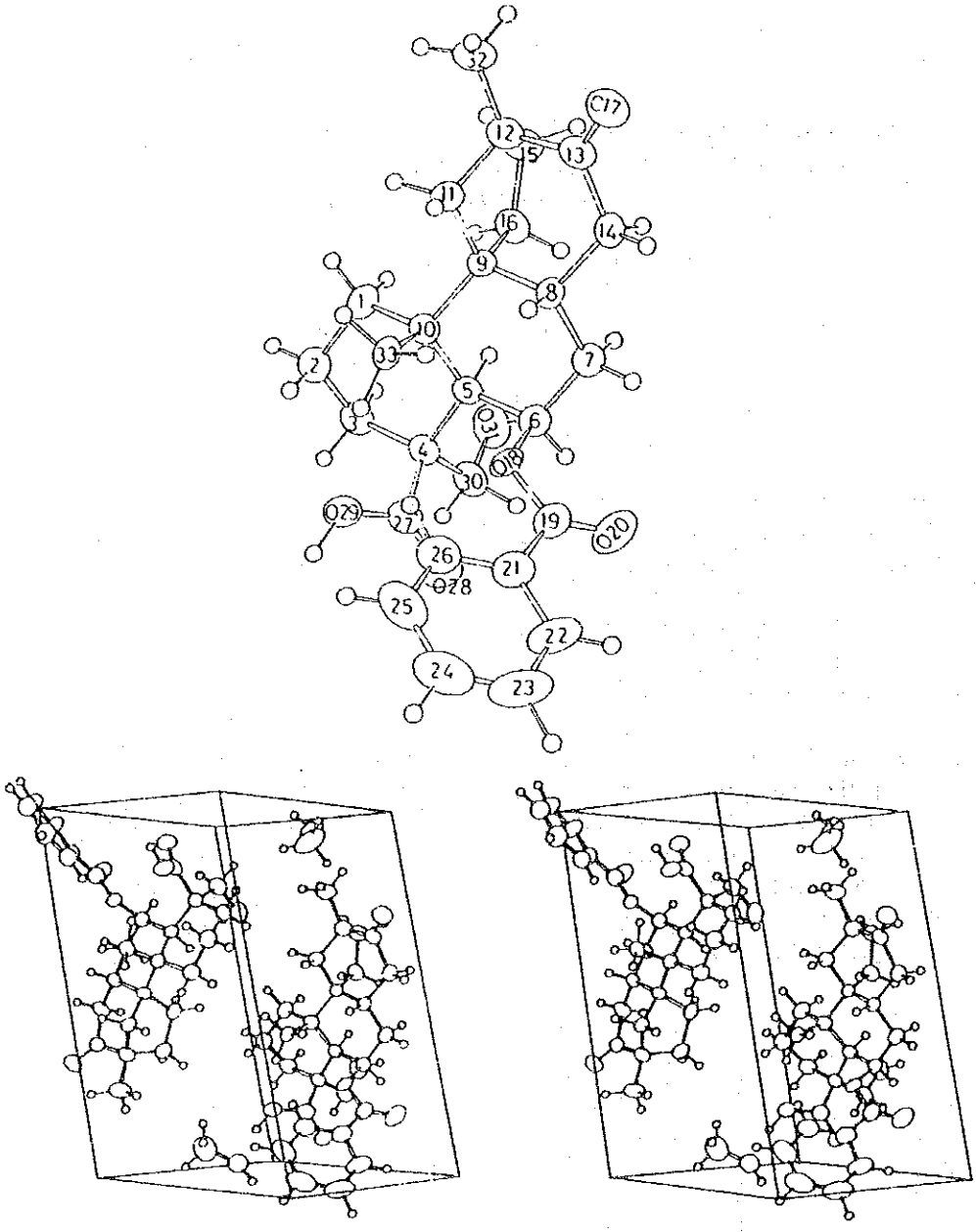


FIGURE 1. Crystal structure of scopadulcic acid A [**4**] and its molecular packing.

extracted with 70% EtOH at boiling temperature (1 h, 3 times). The extract (333 g) was suspended in H_2O and extracted with *n*-hexane. The part that was insoluble in both solvents (67.1 g) was filtered off and was further extracted with $CHCl_3$. The $CHCl_3$-soluble part (54.3 g) was repeatedly chromatographed on a Si gel column ($CHCl_3$/MeOH and *n*-hexane/EtOAc) to afford 130 mg of scopadulcic acid A [**4**].

SCOPADULCIC ACID A [**4**].—$C_{27}H_{34}O_6$, mp 172–174° (MeOH), $[\alpha]D$ −5.7° (MeOH), ^{13}C nmr (100.4 MHz, Me_2CO-d_6) δ 35.1 (C-1), 20.1 (C-2), 33.5 (C-3), 48.8 (C-4), 45.0 (C-5), 70.4 (C-6), 35.8 (C-7), 37.0 (C-8), 54.2 (C-9), 40.0 (C-10), 46.5 (C-11), 53.2 (C-12), 212.8 (C-13), 43.5 (C-14), 37.7 (C-15), 24.6 (C-16), 20.5 (C-32), 68.2 (C-30), 178.3 (C-27), 21.6 (C-33), 166.8 (C-19), 132.5 (C-21), 130.8 (C-22, C-26), 129.5 (C-23, C-25), 133.7 (C-24).

CRYSTALLOGRAPHIC ANALYSIS OF **4**.—Scopadulcic acid A [**4**] was analyzed as its MeOH solvate. A crystal of dimensions 0.5 × 0.5 × 0.2 mm was selected for X-ray measurements. Crystal data: $C_{27}H_{34}O_6$·MeOH, M_w 486.6, mono-

TABLE 1. Fractional Atomic Coordinates ($\times 10^4$) and Temperature Factors ($Å^2 \times 10^3$) for Non-hydrogen Atoms of Scopadulcic Acid A [4].

Atom	x	y	z	U_{eq}
C-1	5512(5)	2990	5041(7)	45(2)
C-2	6372(5)	3130(9)	6364(7)	46(2)
C-3	7097(5)	2000(9)	6309(7)	40(2)
C-4	7458(5)	1940(7)	4872(7)	32(2)
C-5	6629(5)	2170(7)	3474(7)	31(2)
C-6	6971(5)	2271(7)	2030(8)	35(2)
C-7	6155(5)	2147(8)	677(7)	36(2)
C-8	5311(4)	3072(7)	731(7)	30(2)
C-9	4957(4)	2943(7)	2178(7)	28(2)
C-10	5825(5)	3231(8)	3561(7)	33(2)
C-11	4116(5)	3952(8)	2081(7)	35(2)
C-12	3286(5)	3291(8)	956(8)	40(2)
C-13	3526(5)	3378(7)	−558(8)	35(2)
C-14	4489(5)	2789(9)	−631(7)	40(2)
C-15	3378(5)	1758(9)	1491(9)	46(3)
C-16	4455(5)	1502(8)	2198(8)	38(2)
O-17	2994(4)	3872(7)	−1626(6)	50(2)
O-18	7469(3)	3589(5)	2034(5)	35(2)
C-19	8164(5)	3625(9)	1262(8)	44(2)
O-20	8302(4)	2690(7)	493(7)	75(2)
C-21	8720(5)	4912(9)	1502(8)	44(2)
C-22	9480(6)	5056(11)	815(11)	68(3)
C-23	10041(6)	6191(13)	1026(11)	82(4)
C-24	9845(6)	7238(12)	1919(11)	70(4)
C-25	9100(7)	7130(11)	2604(10)	66(3)
C-26	8536(5)	5964(9)	2399(9)	49(3)
C-27	8313(5)	2919(9)	4963(7)	40(2)
O-28	9012(3)	2646(6)	4497(6)	52(2)
O-29	8231(3)	4131(6)	5614(6)	48(2)
C-30	7886(5)	490(9)	4803(8)	45(3)
O-31	7191(4)	−563(6)	4717(6)	55(2)
C-32	2316(5)	3935(10)	942(9)	56(3)
C-33	6129(5)	4754(7)	3540(7)	33(2)
O-34	373(4)	889(7)	3717(7)	68(2)
C-35	781(9)	1916(15)	3061(14)	111(6)

clinic, space group $P2_1$, a = 14.355(2), b = 9.732(2), c = 9.297(2) Å, β = 103.30(1)°, V = 1263.9(4) $Å^3$, Z = 2, D_x = 1.28 $Mg \cdot m^{-3}$, μ(MoKα) = 0.977 cm^{-1}. Data were collected by a Rigaku AFC-5 diffractometer equipped with graphite monochromated MoKα radiation (λ = 0.7107 Å) and 2θ-ω scan mode. Unit cell parameters were determined by least-squares fit of 47 strong reflections (29° ≦ 2θ ≦ 37°). Of the 2521 reflections measured (3° ≦ 2θ ≦ 50°), 2367 were unique and 1786 with Fo ≧ 2σ(Fo) were considered observed and used in the calculations. The crystal structure was solved by direct methods using MULTAN 78 (4) and refined by full-matrix least-squares on F using unit weight (5). The positions of H atoms were calculated from an idealized geometry and checked with the D maps. Convergence was achieved with R = 0.063. For the fractional atomic coordinates and the temperature factors for non-hydrogen atoms of **4** see Table 1.[1]

ACKNOWLEDGMENTS

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8-HYDROXYTRICETIN 7-GLUCURONIDE, A β-GLUCURONIDASE INHIBITOR FROM SCOPARIA DULCIS

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($CDCl_3$): δ 7.70–7.90 (*m*, 2H), 7.40–7.60 (*m*, 3H), 6.73 (*s*, 1H), 6.64 (*s*, 1H), 6.05 (*s*, 2H), 4.12 (*s*, 3H); ^{13}C NMR ($CDCl_3$): δ 177.5 (*s*, C=O, C-4), 160.8 (*s*, C-2 or C-9), 154.7 (*s*, C-2 or C-9), 152.9 (*s*, C-5), 141.4 (*s*, C-7), 134.9 (*s*, C-6), 131.4 (*s*, C-1'), 131.2 (*d*, C-4'), 128.9 (*d*, C-2' or C-6'), 125.9 (*d*, C-3' or C-5'), 112.9 (*s*, C-10), 108.3 (*d*, C-3), 102.1 (*t*, O-CH_2-O), 93.2 (*d*, C-8), 61.1 (*q*, OMe); EIMS *m/z* (rel. Intensity): 296 (M^+ 34.1), 268 (55.5, M-CO), 250 (54.8), 222 (10.6), 237 (10.6), 194 (10.1), 166 (100), 164 (96.7), 136 (33.9), 105 (17.8), 102 (62.1).

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8-HYDROXYTRICETIN 7-GLUCURONIDE, A β-GLUCURONIDASE INHIBITOR FROM *SCOPARIA DULCIS*

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Key Word Index—*Scoparia dulcis*; Scrophulariaceae; flavonoids, 5,7,8,3',4',5'-hexahydroxyflavone 7-*O*-β-D-glucuronide; β-glucuronidase inhibitor.

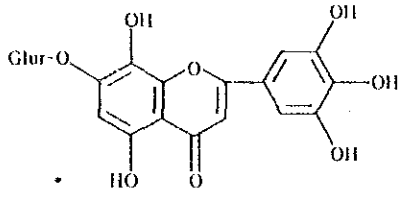
Abstract—A new flavone glycoside has been isolated from *Scoparia dulcis* together with 11 known compounds. The new glycoside was determined as 5,7,8,3',4',5'-hexahydroxyflavone glucuronide by spectral analysis. The new glycoside and isovitexin showed inhibitory activity against β-glucuronidase.

INTRODUCTION

Scoparia dulcis L. is a perennial herb which has been used for the treatment of stomach disease and hepatosis in Paraguay, as a cure for hypertension in Taiwan [1, 2] and for toothache, blennorrhagia and stomach problems in India [3]. From Indian *S. dulcis*, an antidiabetic compound named amellin was isolated by Nath [4]. Earlier phytochemical studies on this medicinal plant resulted in isolation of hexacosanol, D-mannitol, sitosterol [3] and 6-methoxybenzoxazolinone [5] as well as triterpenoids [5, 6] and flavonoids [7]. Previously, we reported the isolation and structural elucidation of five new diterpene acids from the 70% ethanolic extract of a plant collected in Paraguay [8–10]. In a continuation of this work, we have examined the water-soluble fraction which showed mild inhibitory activity against β-glucuronidase. This paper deals with isolation of flavonoids from this fraction and their inhibitory activity against β-glucuronidase.

RESULTS AND DISCUSSION

Eleven flavonoids (**1**–**11**) and a phenylpropanoid (**12**) were isolated from the water-soluble fraction of a 70% ethanolic extract of *S. dulcis*. The compounds, **1**–**10**, **12**, were identified as apigenin (**1**), scutellarein (**2**), luteolin (**3**), vicenin-2 (**4**), linarin (**5**), vitexin (**6**), isovitexin (**7**), scutellarin (**8**), scutellarin methyl ester (**9**), luteolin 7-glucoside (**10**) and *p*-coumaric acid (**12**) by direct comparison of their physical and spectroscopic properties (mp, IR, UV, ^1H NMR and ^{13}C NMR) with those of authentic samples,



11

respectively. Compounds 1, 2 and 3 are considered to be artifacts from their respective glycosides.

Compound 11 *gave positive colour reactions with* Mg/HCl and $FeCl_3$. The UV spectrum exhibited the characteristic absorptions of a flavonoid skeleton at 348 nm (band I) and 275 nm (Band II) [11]. A large bathochromic shift of band I of 87 nm was observed in the presence of $AlCl_3$ suggesting the presence of free *ortho*-dihydroxyl groups in the B-ring. Addition of HCl to this solution caused a hypsochromic shift of Band I of 75 nm, but bathochromic shifts of Band I and Band II still remained relative to the original spectrum indicating the presence of a chelated hydroxyl group at C-5 [11]. No *bathochromic shift of Band II* was observed with sodium acetate indicating the absence of a free hydroxyl group at the C-7 position. The UV spectrum exhibited a bathochromic shift of Band I upon addition of NaOMe suggesting the presence of a free 4'-hydroxyl group. The ^{1}H NMR spectrum revealed five hydroxyl protons (δ12.41, 1H; 9.46, 2H; 9.15, 1H; 8.74, 1H) and four aromatic protons (δ6.62, *s*, 1H; 6.66, *s*, 1H; 7.09, *s*, 2H) indicating oxygenation at C-3', C-4', C-5', C-5, C-7 and C-6 or C-8. The A ring substitution pattern of 11 was determined by comparison of its ^{13}C NMR spectrum with those of scutellarin (8) and isoscutellarein 4'-methylether 7-*O*-[2''-*O*-(6'''-acetyl)-β-D-allopyranosyl]-β-D-glucopyranoside (13) [12, 13]. The chemical shifts of the carbons on the A-ring of 11 were similar to those observed for the A-ring of 13 indicating a 5,7,8-substitution *of the A-ring in 11. (See Table 1). The* ^{13}C *NMR* spectrum also showed signals due to a glucuronic acid moiety [14].

Table 1. δ values in ^{13}C NMR spectrum of compounds 11, a 5,6,7-substituted flavone (8) and a 5,7,8-substituted flavone (13) in DMSO-d_6

Position	11	13	8
2	164.39 *s*	163.4	164.03 *s*
3	102.66 *d*	103.3	102.44 *d*
4	182.13 *s*	182.2	182.28 *s*
5	150.48 *s*	150.5	146.76 *s*
6	98.00 *d*	99.4	130.35 *s*
7	151.90 *s*	152.0	148.93 *s*
8	126.80 *s*	127.6	93.47 *d*
9	144.47 *s*	143.7	150.88 *s*
10	105.20 *s*	105.5	105.80 *s*

8 = *scutellarin*, 11 = *isoscutellarein 4'-methylether* 7-*O*-[2''-*O*-(6'''-acetyl)-β-D-allopyranosyl-β-D-glucuronide [12, 13].

From the coupling constant (6.6 Hz) of the anomeric proton appearing at δ 5.17 in the ^{1}H NMR spectrum of 11, a β-linkage of glucuronic acid at C-7 was suggested. In the EIMS spectrum of 11, a fragment ion peak at *m/z* 318 (M^+ – glucuronic acid) was observed and its elemental composition determined as $C_{15}H_{10}O_8$ by HRMS. The fragment ion peaks at *m/z* 169 and 150 correspond to the A- and B-rings, respectively. Thus, 11 was elucidated as 5,7,8,3',4',5'-hexahydroxyflavone 7-*O*-β-D-glucuronide.

The inhibitory activities of the isolated flavonoids (1–11) against β-glucuronidase were measured according to Nobunaga's method with a slight modification [15]. The results are shown in Table 2. Of 11 flavonoids, only isovitexin (7) and 11 showed mild inhibitory activity against β-glucuronidase from bovine liver. The inhibitory activity of 11 was one-tenth of that of the well known β-glucuronidase inhibitor, glucosaccharo-1:4-lactone. Kinetic studies were conducted with 11 in order to determine the type of inhibition. The Lineweaver–Burk plots of the inhibition by 11 are given in Fig. 1, and from these results 11 *seems to be a mixed-type inhibitor of* β-*glucuronidase*. The inhibitory effect of 11 on β-glucuronidase was also examined in the presence of a large amount of bovine serum albumin (BSA) (100–400 times). In these conditions 11 showed almost the same degree of inhibition in the presence of 400 times of BSA, suggesting that it inhibits the activity of β-glucuronidase even in the presence of other proteins.

EXPERIMENTAL

General. All mps: uncorr. The ^{1}H NMR spectra were taken at 270.05 MHz and the ^{13}C NMR spectra were measured at 50.3 MHz in DMSO-d_6 solutions, and chemical shifts are given in δ(ppm) with TMS as int. standard. The EIMS spectra were obtained at 70 eV.

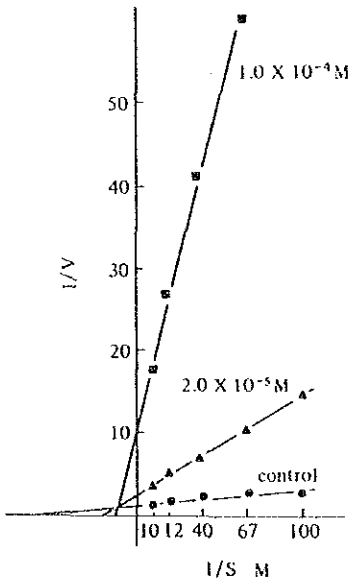


Fig. 1. Inhibitory effects of 5,7,8,3',4',5'-hexahydroxyflavone 7-*O*-β-D-*glucuronide on* β-*glucuronidase* (*Lineweaver–Burk* plots). 1/V: [1/(optical density at 405 nm)] × 1000; 1/S: 1/(concentration of *p*-nitrophenyl-β-D-glucuronide).

Plant material. Scoparia dulcis L. was collected in March 1985, near Asunción, Paraguay. Voucher specimens have been deposited in the herbarium of herbal garden, Toyama Medical and Pharmaceutical University.

Extraction and isolation of flavonoids. Dried whole plants of *S. dulcis* (2 kg) were ground and extracted with hot 70% EtOH × 3. The extract was coned *in vacuo* and the residue still containing water was freeze-dried to give a brown powder (118 g) a 100 g of which was partitioned between H_2O and *n*-hexane and the aqueous layer extracted successively with $CHCl_3$, Et_2O, EtOAc–MeOH (9:1) and EtOAc–MeOH (4:1). The Et_2O extract (1.7 g) was run on a polyamide column followed by separation on a Sephadex LH-20 column to give 3 flavonoids, **1** (7.4 mg), **2** (9.1 mg), **3** (5.1 mg) and **12** (18.3 mg). The EtOAc–MeOH (9:1) extract was fractionated by a combination of polyamide CC, prep. TLC (cellulose) and Sephadex LH-20 CC to afford **4** (trace), **6** (16.3 mg), **9** (20.9 mg) and **10** (6.5 mg). The EtOAc–MeOH (4:1) extract was run on a polyamide column eluted successively with H_2O–MeOH, Me_2CO and DMF. The fractions eluted with H_2O, 50–60% MeOH, 60–80% MeOH and DMF were further subjected to cellulose PLC and Sephadex LH-20 CC to afford **4** (541.4 mg), **5** (15.3 mg), **6** (7.1 mg) and **7** (20.8 mg), and **8** (472.6 mg) and **11** (32.2 mg), respectively.

5,7,8,3',4',5'-Hexahydroxyflavone 7-O-β-D-glucuronide (**11**). Yellow needles from MeOH–H_2O, mp > 300°. UV λ_{max}^{MeOH} nm 252sh, 275, 315sh, 348; + NaOMe 300sh, 380sh (dec.); + $AlCl_3$ 238sh, 278, 328, 435, + $AlCl_3$ + HCl 280, 330sh, 360, + NaOAc 260sh, 335, 412, + NaOAc + H_3BO_3 260sh, 310sh, 483. EIMS *m/z*: 318 (71), 169 (16), 150 (8). HRMS: found, 318.0372 (M^+ − glucuronic acid); calcd. for $C_{15}H_{10}O_8$, 318.0372. ^{1}H NMR (DMSO-d_6): δ 12.41 (1H, *s*, OH), 9.46 (2H, *s*, OH), 9.15 (1H, *s*, OH), 8.74 (1H, *s*, OH), 7.09 (2H, *s*, H-2',6'), 6.66 (1H, *s*, H-3), 6.62 (1H, *s*, H-6), 5.17 (1H, *d*, *J* = 6.6 Hz, H-1''). ^{13}C NMR (DMSO-d_6): δ 164.39 (*s*, C-2), 102.66 (*d*, C-3), 182.13 (*s*, C-4), 150.48 (*s*, C-5), 98.00 (*d*, C-6), 151.90 (*s*, C-7), 126.80 (*s*, C-8), 144.47 (*s*, C-9), 105.20 (*s*, C-10), 120.40 (*s*, C-1'), 105.73 (*d*, C-2'), 146.04 (*s*, C-3'), 137.82 (*s*, C-4'), 146.04 (*s*, C-5'), 105.73 (*d*, C-6'), 100.33 (*d*, C-1''), 72.69 (*d*, C-2''), 74.80 (*d*, C-3''), 71.14 (*d*, C-4''), 75.22 (*d*, C-5''), 169.91 (*s*, C-6'').

Determination of β-glucuronidase activity. β-Glucuronidase activity was determined by measuring the absorbancy at 405 nm of *p*-nitrophenol formed from the substrate by the method of Nobunaga [15] with the following modification. The reaction mixture contained 0.9 ml of 0.1 M acetate buffer (pH 5.0), 0.03 ml of 0.1 M *p*-nitrophenyl-β-D-glucuronide and 0.1 ml of adequately diluted enzyme soln was incubated at 37° for 20 min. After addition 0.25 ml of 0.2 M Na_2CO_3 to stop the reaction, the absorbancy at 405 nm was measured. The inhibitory activity was determined as described for the assay of enzyme activity, except that test material dissolved in 0.1 M acetate buffer (pH 5.0) was mixed with substrate and enzyme. The inhibitory activity (%) was calculated as follows: $(E - S)/E \times 100$, where E is the activity of enzyme without test material and S is the activity of enzyme with test material.

Inhibitory activities of flavonoids on β-glucuronidase. Compound (IC_{50}): **1** (> 10^{-4}M), **2** (> 10^{-4}M), **3** (> 10^{-4}M), **4** (> 10^{-4}M), **5** (> 10^{-4}M), **6** (> 10^{-4}M), **7** (4.6×10^{-5}M), **8** (> 10^{-4}M), **9** (> 10^{-4}M), **10** (> 10^{-4}M), **11** (1.8×10^{-5}M), glucosaccharo-1:4-lactone (1.8×10^{-6}M, control).

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<添付論文 13>

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PENTAGALLOYLGLUCOSE, A XANTHINE OXIDASE INHIBITOR FROM A PARAGUAYAN CRUDE DRUG, "MOLLE-I" (*SCHINUS TEREBINTHIFOLIUS*)

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As a part of our continuing search for biologically active substances from medicinal plants in Paraguay, we have examined inhibitory activities of 70% EtOH extracts of about 60 native crude drugs against xanthine oxidase (XO, EC 1.2.3.2). We report here that the extract of the aerial parts of Paraguayan "Molle-i" (*Schinus terebinthifolius* Raddi, Anacardiaceae) showed significant inhibitory activity (79.8% at 50 μg/ml) against cow's milk xanthine oxidase. Flavonoids (1–5), hydroxychalcones (1), coumarins (1), 2,8-dihydroxyadenine (6), and xanthones (7) have previously been reported as naturally occurring inhibitors of XO. An inhibitor of XO could potentially be useful as a therapeutic agent for hyperuricemia that causes gout (8), renal stones (8,9), or ischemic myocardium (10).

Bioassay-directed fractionation of the extract resulted in the isolation of an active substance as a colorless, amorphous powder. The compound was positive in the $FeCl_3$ reaction and showed absorption bands at 280 nm and 3400, 1720, and 1605 cm^{-1} in the uv and ir spectra, respectively. The ^{1}H-nmr spectrum indicated the presence of oxymethylene protons (δ 4.42, dd, J = 4.4, 11.8 Hz; 4.54, d, J = 11.8 Hz), of five oxygenated methine protons (δ 4.54, m; 5.61, dd, J = 9.8, 8.3 Hz; 5.66, t, J = 9.8 Hz; 6.00, t, J = 9.8 Hz; 6.33, d, J = 8.3 Hz), and of ten aromatic protons (δ 6.97, 7.01, 7.05, 7.11, 7.18, each 2H, s), which were characteristic of pentagalloylglucose (11). The compound was identified as 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose by direct comparison (tlc, ir, ^{1}H nmr, [α]D) with an authentic sample.

The inhibitory activity against XO of pentagalloylglucose was then compared with that of allopurinol, a well known XO inhibitor (12). As shown in Table 1, pentagalloylglucose exhibited the same level of activity as allopurinol. Kinetic analysis of the reaction of XO with pentagalloylglucose by Lineweaver-Burk plots revealed that it inhibited XO noncompetitively at the concentration of 2.12×10^{-6} M. Inasmuch as pentagalloylglucose was reported to inhibit sialidase, hyaluronidase, alkaline phosphatase, and cholesterol oxidase (13), the inhibitory activity might be due to its tannic character. On the other hand, pentagalloylglucose was found to exert an antiherpetic (antiviral) effect (14). This is the first reported isolation of 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose from *S. terebinthifolius*.

TABLE 1. Inhibitory Activity of Pentagalloylglucose and Allopurinol Against Cow's Milk Xanthine Oxidase.

Compound	IC_{50}(M)
Pentagalloylglucose	3.2×10^{-6}
Allopurinol	1.6×10^{-6}

EXPERIMENTAL

PLANT MATERIAL.—Aerial parts of *S. terebinthifolius* were collected at Itapua, Paraguay in Oc-

tober 1985. The voucher specimens are deposited in the Herbal Garden of the Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University and Seccion Botanica, Facultad de Ciencias Químicas, Universidad Nacional de Asunción.

EXTRACTION AND ISOLATION OF PENTAGALLOYLGLUCOSE.—The aerial parts of air-dried *S. terebinthifolius* (100 g) were ground to a fine powder and extracted with 70% EtOH at boiling temperature (1h, 3 times) to give a brownish powder (36 g). A portion of this extract (8.4 g) was partitioned between H_2O and *n*-hexane, and the aqueous layer was successively extracted with $CHCl_3$ and *n*-BuOH. The *n*-BuOH layer, the most active fraction (84.0% at 50 μg/ml), was chromatographed on a column of Sephadex LH-20. Elution of the column with MeOH afforded a mixture of pentagalloylglucose and an unknown compound which were separated by preparative layer chromatography [Si gel, C_6H_6-HCO_2H-ethyl formate (2:2:7)]. A colorless, amorphous powder (7 mg) was obtained and identified as 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose by direct comparison with an authentic sample.

ASSAY OF XANTHINE OXIDASE ACTIVITY.—Cow's milk xanthine oxidase was purchased from Boehringer Mannheim. Xanthine was obtained from ICN Pharmaceutical. All other chemicals were of analytical grade. Xanthine oxidase activity was measured by the method reported by Noro *et al.* (2). The inhibitory activity (%) was calculated by the formula $(1 - B)/A \times 100$, where A is the activity of the enzyme without test material and B is the activity of the enzyme with test material.

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