PN ABU-410
93210
9.334

FINAL REPORT
(April 30,1990- April 30,1994)

Submitted to the Office of the Science Advisor
U.S. Agency for International Development

Enzyme-linked Immunoassay for the Determination of Bruceine and Related Quassinoid compounds in *Brucea javanica* (L) Merr.

Principal Investigator : Dr. Porntip Chaimanee
Institution : Department of Chemistry, Faculty of Science
Silpakorn University, Nakorn Pathom 73000
Thailand Tel 6634-255797, Fax 6634-255820
Email Porntip @ cc.snamcn.su.ac.th

Project Number : 9.334
Grant No. 493-5542-G-SS-0020-00

A.I.D. Grant Project Officer : Dr. Peter Deiken

REC'D IN R&D/R
MAR 14 1995

Project Duration : April 30, 1990- April 30, 1994

FINAL REPORT
(April 30,1990- April 30,1994)

Submitted to the Office of the Science Advisor
U.S. Agency for International Development

Enzyme-linked Immunoassay for the Determination of Bruceine and Related Quassinoid compounds in *Brucea javanica* (L) Merr.

Principal Investigator : Dr. Porntip Chaimanee
Institution : Department of Chemistry, Faculty of Science
Silpakorn University, Nakorn Pathom 73000
Thailand Tel 6634-255797, Fax 6634-255820
Email Porntip @ cc.snamcn.su.ac.th

Project Number : 9.334
Grant No. 493-5542-G-SS-0020-00

A.I.D. Grant Project Officer : Dr. Peter Deiken

Project Duration : April 30, 1990- April 30, 1994

Table of Contents

		Page
Research Objectives		1-3
Methods and Results		
Part A	Extraction and Purification of bruceolides and related compound	4-10
Part B	Development of an enzyme linked immunoassay for bruceolides compound	11-18
Impact, Relevance and Technology Transfer		19
Project Activities/Outputs		19
Project Productivity		19
Future Work		20
Literature Cited		21-22

Research Objectives

The number of malaria cases is raising. Some 280 million people are infected with malaria parasites and at least 110 million develop the disease each year. Drug-resistant malaria parasite now affect almost every country where the disease is endemic. In parts of Thailand, for example, half of the cases of malaria are resistant to treatment with mefloquine. Chloroquine is now all but useless in many countries. Antimalarial prophylaxis and therapy are no longer straightforward because drug resistance has become rampant (Wellems 1991).

This condition has triggered off mass screening of new compounds, either synthetic or from plants, for potential antimalarial activity (Wernsdorfer and Kousnetov 1980). Recent studies of numerous Simaroubaceae plants have highlighted good antimalarial activity of certain quassinoids against chloroquine-resistant strains of *Plasmodium falciparum* (Trager and Polonsky 1981, Guru et al. 1983, O'Neill et al. 1986). *Brucea javanica* (L.) Merr. is wildly distributed throughout Asia, where the bitter fruits are used in traditional medicine for various ailments including malaria (Key 1976, Long-ze et al. 1982, O'Niell et al. 1987) , amoebic dysentery (Killin et al. 1982, Keene et al. 1986) and cancer (Phillipson and Darwish 1979, Pierre et al. 1980). The active principles isolated are a type of quassinoids which is based on the parent pentacyclic alcohol bruceolide (Figure 1).

Studies of the structure activities relationship by variation of substituents in bruceolides have established the structural requirement of optimal antimalarial activity. The presence of C-15 ester function is essential in antimalarial activity, where as unsaturated bond in C-15 ester

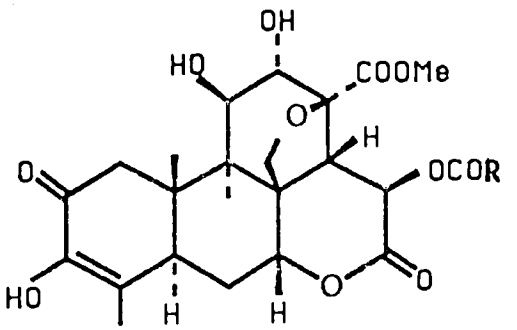
also enhances the activity (Table I).

In view of the the widespread distribution of Brucea species and possible existence of different chemical strains, *B. javanica* (L.) Merr. fruits planted in Thailand is reinvestigated for bruceolides and other novel quassinoids.

A chemical synthesis of this bruceolide is up to now not possible. Therefore the supply of this compound depends on the natural resources of the plant material. Beside this, the biotechnological production of the compound by high-yielding cell cultures may be an alternative method to increase the production of this compound.

For these investigations, a highly specific and sensitive method is necessary which allows the rapid and precise determination of bruceolide compound even in crude plant extract. Therefore an enzyme linked immunoassay (EIA) for detection of high potential antimalarial drug, bruceolides, will be developed.

Furthermore, the panel of antibodies developed for each bruceolide will open up a defined antigenic structure by cross-reactivities studies. This information is essential for the study of bruceolides drug mechanism.



Compound	R
bruceantin	CH=C(Me)CH(Me)$_2$
bruceantinol	CH=C(Me)C(OAc)(Me)$_2$
brusatol	CH=C(Me)$_2$
bruceine A	CH2CH(Me)$_2$
bruceine B	Me
bruceine C	CH=C(Me)C(OH)(Me)$_2$

Figure 1. Structure of bruceolides isolated from *Brucea javanica* fruits.

Table I. In vitro antimalarial and cytotoxic activities of bruceolides.*

Bruceolide	Antimalarial activity in vitro IC 50 (μ g/ml)	Cytotoxic activity in vitro ED 50 (μ g/ml)
bruceantin	0.0008	0.007
brusatol	0.003	0.11
bruceine A	0.011	0.031
bruceine B	0.011	nd
bruceine C	0.005	nd

*Adapted from Phillipson and Darwish 1979, O'Niell et al. 1986 and 1987

Methods and Results

Part A Extraction and Purification of bruceolides and related compounds

General experimental procedures

High resolution mass spectra were run on a VG 7070 F Micromass spectrometer at 70 eV with a source temperature of 200°C, using the direct insertion technique. ^{1}H-nmr spectra were recorded in $CDCl_3$ or DMSO-d_6 on the Bruker AM 300 and Bruker AMK 400 (400 MHz) with TMS as an internal standard. Infrared spectra were performed on a JASCO 302A spectrophotometer. PTLC was performed on Merck silica gel G F254, TLC was carried out on Merck precoated Kieselgel 60 F254 plates with thickness of 0.25 nm. Hplc was performed on a μ -bondapak C-18, 10 mm column (7.8 x 300 mm). Melting points were determined on the Electrothermal melting Point apparatus and are uncorrected.

Plant material : *Brucea javanica* L. (Merr) fruits were purchased from drug stores and identified by associated professor Witaya Pongmala, Biology department, Silpakorn University.

Extraction and Purification

Extraction : The fruits were grounded and successively extracted with hexane, chloroform and methanol. The concentrated methanol extract was partitioned between $CHCl_3$, *n* BuOH and H_2O. The crude extracts were tested for in vitro antimalarial activity against a multi-drug resistant strain of *P. falciparum.*

Separation and purification : The chloroform extract and chloroform-soluble extract (from MeOH) was subjected to a column chloromatography eluted with 1-40% $CHCl_3$-MeOH gradient. Fractions (100 ml) were collected and fractions of similar TLC were obtained. Three fractions given greyish-black to $FeCl_3$ were obtained. Further separation was performed on a silica gel column eluted with 10-50% MeOH-Benzene. Fractions (25 ml) were collected. Bruceine A was combinded as colourless needles after recrystallization from $CHCl_3$-MeOH. Other fractions were separated on repeated preparative thin-layer chromatography using 10% MeOH-C_6H_6 or 3% MeOH-$CHCl_3$. Bruceine A (0.0057%) bruceine B (0.00016%) and a coumarinolignan, cleomiscosin A (0.0022%) were obtained.

Characterisation of Bruceolides

Identification of the quassinoid compound Bruceine A and Bruceine B was done by comparison of the m.p. or TLC and spectroscopic data with the authentic samples or with those reported in the literatures.

Bruceine A : m.p. 268-270°C, mixed m.p. with the authentic sample 268-271°C EIMS (high resolution) : m/z 522 ($M^{+\cdot}$ 522.2089, $C_{26}H_{34}O_{11}$ requires 522.2098), 438(60) 297(13), 151(21), 105(22), 85(69). ^{1}H nmr (300 MHz, DMSO) : d 0.99 (6H, d, J = 4.0 Hz, 2 x CH_3); 1.31 (3H, s, C_{10}-CH_{13}), 181 (3H, s, C_4-CH_3); 3.77 (3H, s, OCH_3); 4.55 (1H, d, J = 6.0 Hz, OCH_2); 5.00 (1H, brs, H7) IR (Nujol) ν_{max} :3450 (OH),

1740, 1720 (C=O ester) cm^{-1}

Bruceine B : As bruceine B was obtained in a small amount (3.2 mg) identification was done by compare TLC and ^1H nmr data with the authentic samples given from Dechatwongse, T ^1H nmr (400 MHz, $CDCl_3$-CD_3OH); 1.34 (3H, s, $C_{10}CH_3$); 1.85 (3H, s, C_4CH_3); 2.09 (3H, s, $COCH_3$); 3.79 (3H, s, OCH_3); 4.30 (2H, m, H11, H12); 4.45 (2H, d, J = 7 Hz, OCH_2). TLC was identical with the authentic sample.

Unknown I in the previous report was tentatively identified as Cleomiscosin A, a coumarinolignan.

Cleomiscosin A : m.p. = 264-265 ^oC EIMS (high resolution) m/z 386 ($M^{+\cdot}$ 386.0990, $C_{20}H_{18}O_8$ requires 386.1000), 327 (2.4), 219 (24), 180 (75), 137 (100); ^1H nmr (400 MHz $CDCl_3$-DMSO-d_6, 3:1) d = 3.54 (1H, m, H9′), 3.883, 3.875 (3H each, 2x s, 2x OCH_3), 4.12 (1H, m, H9′), 5.10 (1H, d, J = 4 Hz, H7′), 6.25 (1H, d, J = 4.8 Hz, H_3) 6.87-6.99 (3H, m,H2′, 5′, 6′), 7.71 (1H, d, J = 5.0 Hz, H_4), 8.62 (1H, exchanged with D_2O, OH). ^{13}C nmr (DMSO) δ = 59.6 (OCH_3), 63.9 (C-9′), 79.9 (C-7′), 81.9 (C-8′), 104.9 (C-5), 115..1 (C 1′), 115.3 (C10/, 116.2 (C-2′), 117.2 (C-3), 119.4 (C-5′), 124.8 (C-6′), 130.7 (C 8), 135.7 (C 7), 135.9 (C 9), 142.0 (C6), 148.9 (C-4), 151.3 (C 3′), 151.7 (C 4′), 164.1 (C=O).

IR (Nujol) ν_{max} : 3450 (OH), 1710 (C=O ester) 1620 (C=C aromatic) cm^{-1}

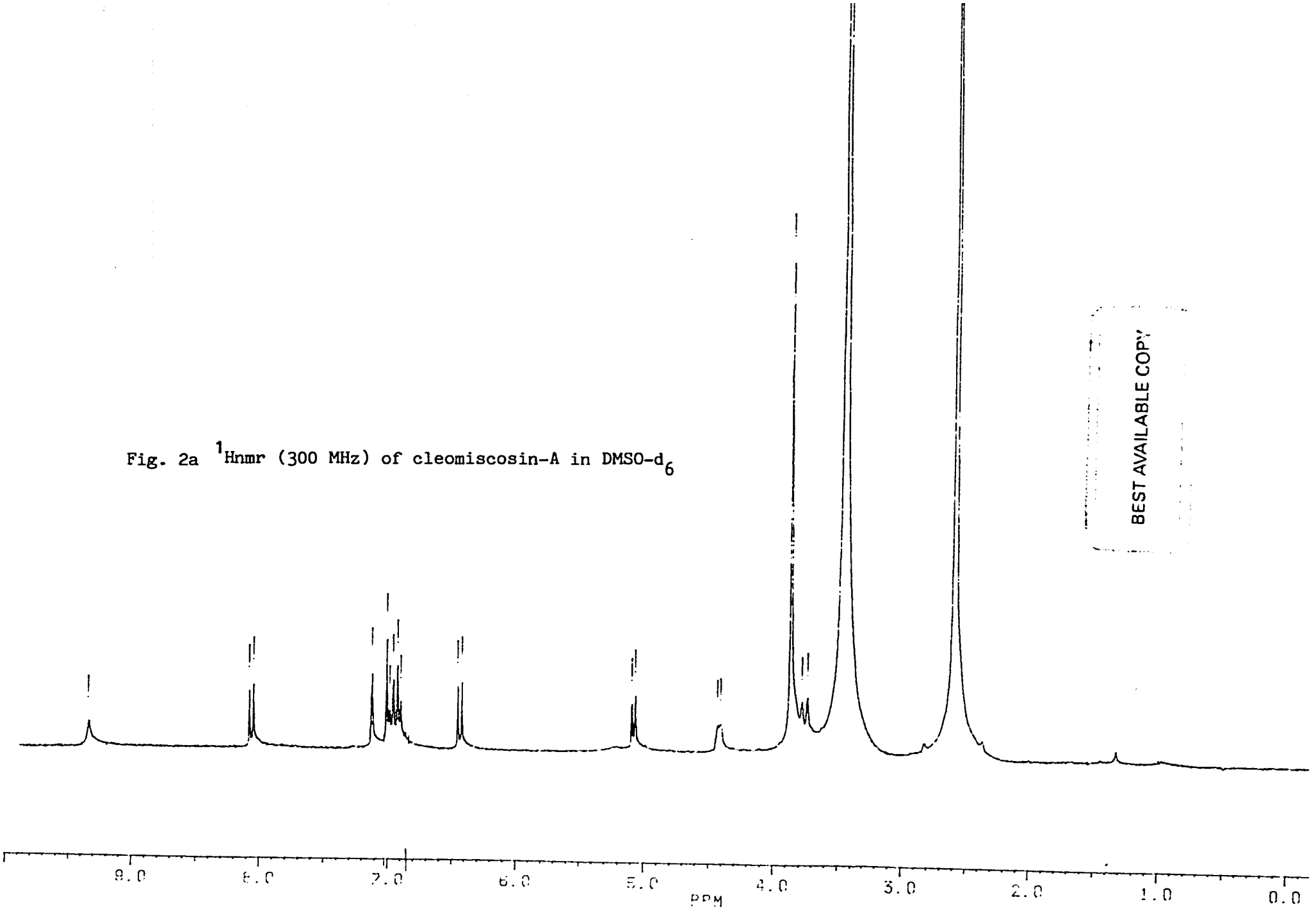
Results and Discussion :

Ten batches of fruits (2 kg, each) were extracted and purified. Bruceine A was the only major quassinoid isolated. Percentage of bruceine A founded was ranging from 0.0030 to 0.0057. Bruceine B (3.2 mg, 0.00016%) was once obtained from one of the fruit (2 kg) extract. The quantity of bruceines presence in the plant depends on the location and season of fruits collection.

The spectroscopic data and physical properties (m.p. and mixed m.p. with the authentic sample) of bruceine A are in accordance with those reported in the literatures (Phillipson and Darwish 1981,Pavanand et al. 1986) However, characterization of bruceine B was acheived by compared the TLC with the authentic sample and the ^1H nmr spectrum was similar to that of reported in the literatures (Phillipson and Darwish 1981, Pavannard et al. 1986).

Together with the quassinoid, a coumarinolignan, cleomiscosin A was also isolated. In TLC (5% MeOH-$CHCl_3$) the R_f clemonisosin A was overlap with that of bruceine A, it was first misunderstood that it was a quassinoid (unknown I, greyish-black to $FeCl_3$). After final purification, it did not give color to $FeCl_3$. The chemical shift (Figure 2a-c) of the methoxy signals in $CDCl_3$-DMSO-d_6 was close together (d, 3.878, 3.875) but in C_5D_5N was quite separate (d, 3.87, 3.83). These values were not correspond with those literature values for clemomisosin A or B (Ray 1982, Arisawa et al. 1984, Arnoldi et al.1984, Tanaka et al. 1985). However, the isolation of cleomiscosin A from *Brucea javanica* was previously reported (Lee et al. 1984). Hence we tentatively identified unknown I as cleomiscosin (A).

Fig. 2a ^{1}Hnmr (300 MHz) of cleomiscosin-A in DMSO-d_6



BEST AVAILABLE COPY

NTP31.005
$CDCl_3$:d_6DMSO
4 : 1
rerun
T = 300°K

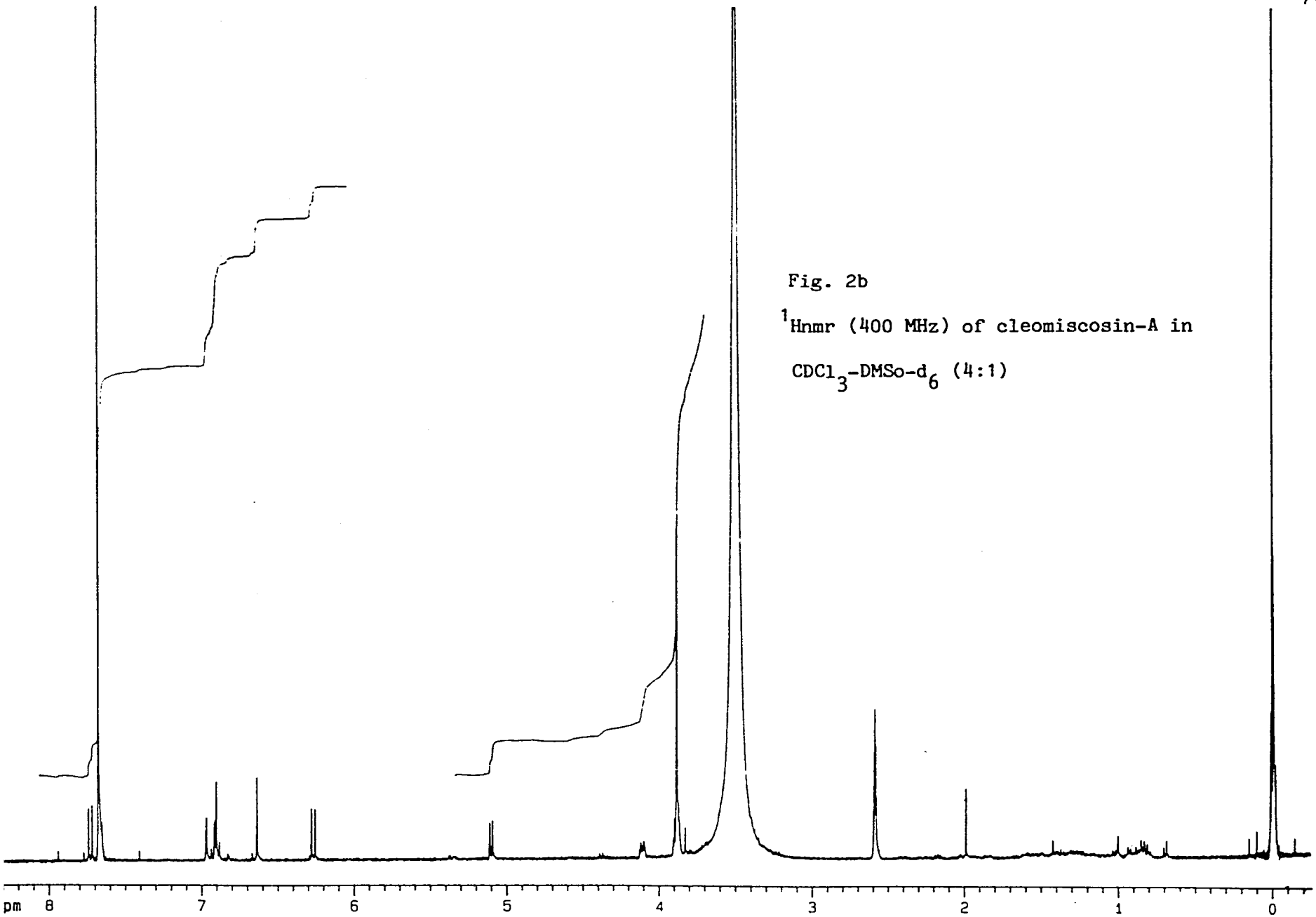


Fig. 2b

^{1}Hnmr (400 MHz) of cleomiscosin-A in

$CDCl_3$-DMSo-d_6 (4:1)

BEST AVAILABLE COPY

NTP31.008
Py 320°K

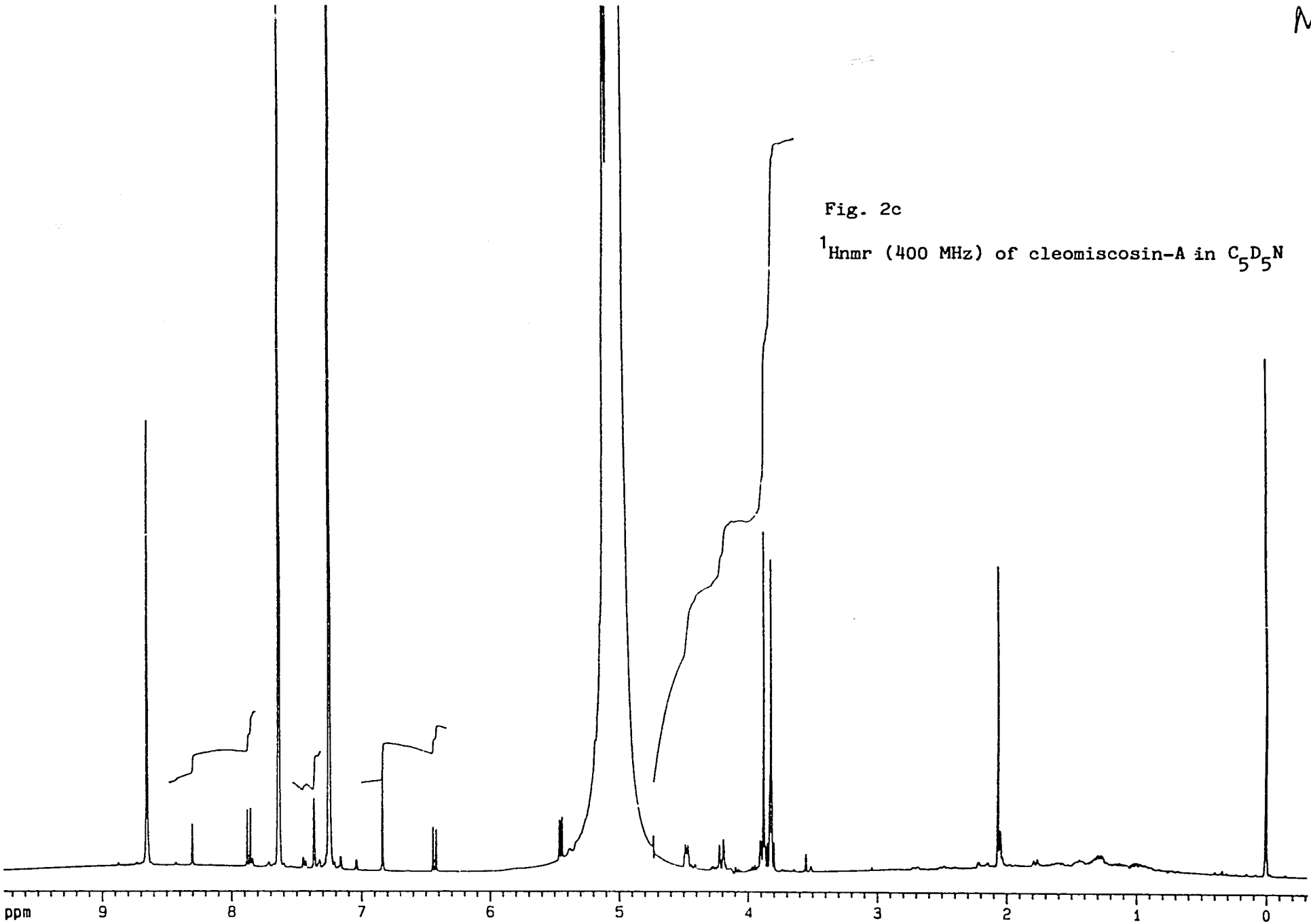


Fig. 2c

^{1}Hnmr (400 MHz) of cleomiscosin-A in C_5D_5N

Part B : Development of enzyme linked immunoassay (EIA) for bruceolides.

Synthesis of succinyl bruceine A :

Bruceine A (60 mg, 109 mmol) and succinic anhydride (60 mg, 600 mmol) were dissolved in 0.4 ml ice cold pyridine. The reaction was carried out under nitrogen and stirring at room temperature. The reaction was mornitored by TLC (Silica gel F_{254} , Merck , Germany; solvent system: chloroform-methanol-triethyamine 9:1:1). The compounds were visualized under UV light,254 nm. The reaction is terminated by addition of 5 ml 1N HCl. The precipitated product was centrifuged at 15,000 rpm 5 min and freeze dried to yield 60 mg of pale yellow powder.

Preparation of succinyl bruceine A - protein conjugates :

Two conjugates were prepared. The conjugate to be used as immunogen was made with bovine serum albumin (BSA) and the coating conjugate with keyhole limpet hemocyanin (KHLH). Both conjugates were achieved by the conjugation of succinyl-bruceine A to the carrier (Figure 3) by the carbodiimide method (Meisner and Meisner 1981)). To 20 mg succinyl bruceine A dissolved in 0.5 ml of ice-cold pyridine were added 1-ethyl-3(3-dimethylamino-propyl) carbodiimide hydrochloride (70 mg in 0.5 ml of 80% pyridine-H_2O) and protein (15 mg in 0.5 ml of H_2O). The solution was stirred for 2h in an ice bath , and then 3 ml of cold acetone and 0.09 ml of 0.5 N HCl were added. The mixture was kept in ice for another hour, and followed by centrifugation. The white

BEST AVAILABLE COPY

BEST AVAILABLE COPY

precipitate was washed twice with 6 ml cold acetone and freeze drying. A molar ratio of 7:1 for succinyl bruceine A : BSA and 18:1 for succinyl bruceine A: KHLH were calculated from the increased absorption at 256 nm of the conjugate over BSA and KHLH respectively (Figure 4).

Immunization and antiserum production :

The succinyl bruceine A-BSA conjugate (1 mg in 1 ml 0.15M NaCl) was administered subcutaneously to 12 week old rabbits as a 1:1 emulsion in Freund's adjuvant once. Booster injection were then given at biweekly intervals, using 1 ml dose of antigen in incomplete Freund's adjuvant at final concentration of 1 mg/ml for 4 weeks vial subcutaneous and intramuscular site thereafter. Blood was collected from the ear veins 10 after each booster injection, and the serum was separated by centrifugation after incubation at 37°C 60 min and stored at -70°C. Bruceine A antibody was demonstrated by Ouchterlony double diffusion in all rabbits immunized with succinyl bruceine A at different titre.

Isolation of antibodies :

The IgG fraction from the antiserum was obtained by ammonium sulfate precipitation as follows : To 4 ml of serum add 16 ml of distilled water and further add 10 ml of saturated $(NH_4)_2SO_4$ solution (pior adjust to pH7.0). The mixture was stirred on ice for 1 h and centrifuged for 10min,4°C at 8000 x g. The precipitate was resuspended in 20ml of water and 10 ml of saturated $(NH_4)_2SO_4$ was again added. After 30 min stirring and centrifugation, the precipitate was resuspended in 20 ml of water and the precipitation procedure repeated two times. The final

BEST AVAILABLE COPY

precipitate was dissolved in 4 ml of half-strength PBS , dialyzed against at least three changes of 1 lit of half-strength PBS for a period of 24 h and lyophilized. The product was stored in -70°C.

Performance of indirect EIA :

Microtiter plates with 96 wells (F-form,Nunc) were used and tripicate determinations were performed throughout. All test solutions were tempered to 37°C before use. After each step of the following test program, wells were washed three times with 300 µl of phosphate buffer saline,pH 7.4, containing 0.5% (v/v)Tween 20 (PBS-Tween). To bind the coating conjugate (succinyl bruceine A-KHLH) on the surface of microtiter plate, 200 µl coating conjugate solution (1 µg dissolved in 0.05M $NaHCO_3$, pH 9.6) was dispensed into each of the 60 inner wells and incubation at 4°C overnight. Thereafter, the coating conjugate solution was discard, and 100 µl of standard solution (free bruceine A) or sample dissolved in PBS-Tween were added to each well. To every well, except those containing 200 µl PBS-Tween (a"no antibody" blank), 100 µl antiserum diluted or IgG fraction in PBS-Tween was added and plates incubated in humid box overnight at 4°C. After this step, the wells were emptied and 200 µl of second antibody solution (alkaline -phosphatase-linked goat anti rabbit immune serum) diluted 1:8000 in PBS-Tween was added to each well. Plates were incubated 3 h at 37°C, emptied and washed. 200 µl fresh solution of 3.8 mM 4-nitrophenylphosphate in 0.5 M $NaHCO_3$ buffer,pH9.6 containing 0.5 mM $MgCl_2$ added per well and the

plates were further incubated at 37°C for 90 min. The enzyme reaction was measured photometrically at 405 nm using Biotek Microplate Reader EIA calculations were performed utilizing immunoassay computation software.

Results and Discussion

General assay parameters :

For the performance of EIA, the optimal assay conditions depend on the quantity of coating antigen-protein conjugate bound to the surface of the microtiter wells as well as on the suitable dilution of antiserum used. For the bruceine A-specific EIA, 1 μg coating antigen-protein conjugate per well combined with a 200 fold dilution of the antiserum or 1 μg of specific IgG gave optimal results (Figure 5 and 6).

Assay sensitivity

The sensitivity of bruceine A-specific EIA is demonstrated by typical standard curves shown. The measuring range of EIA develope with antiserum extended from 80 ng-6000 ng (Figure 5). In case of EIA develpoe with specific IgG, the measuring range of this assay ranged from 50 ng-6000 ng (Figure 6).

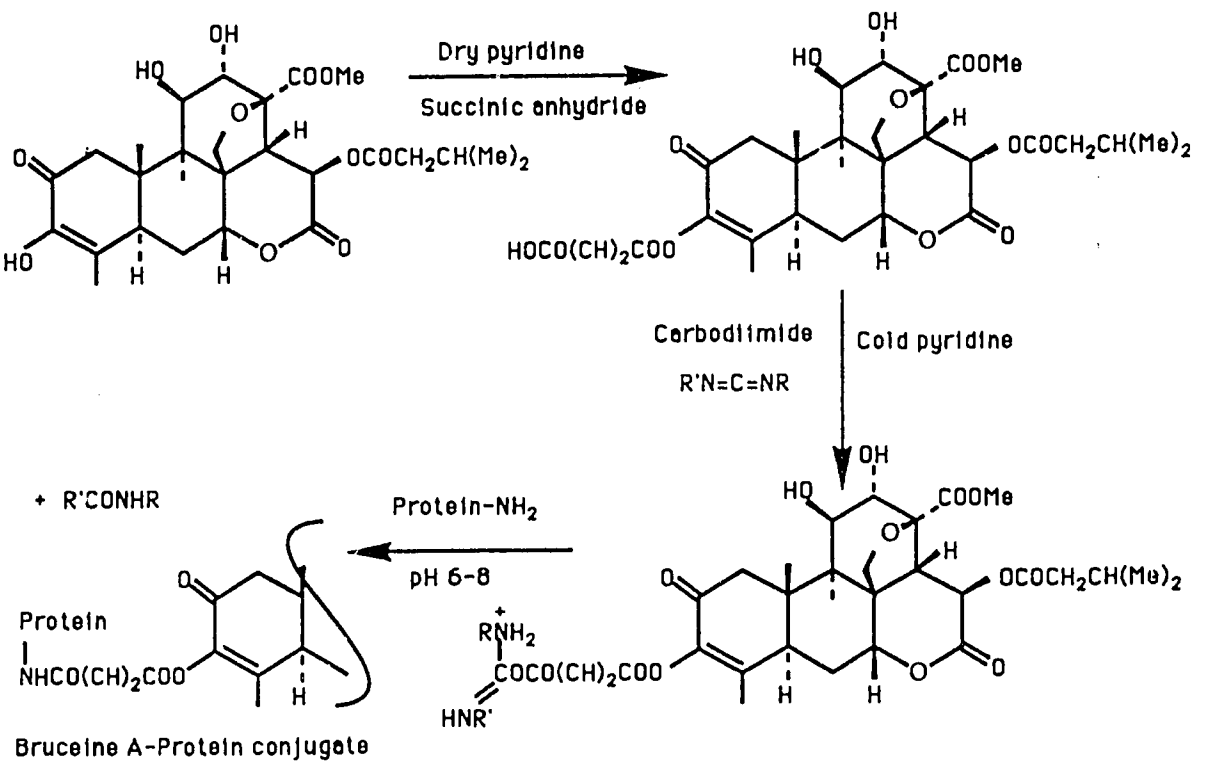


Figure 3. Reaction sequence of preparation of succinyl bruceine A-protein conjugate.

BEST AVAILABLE COPY

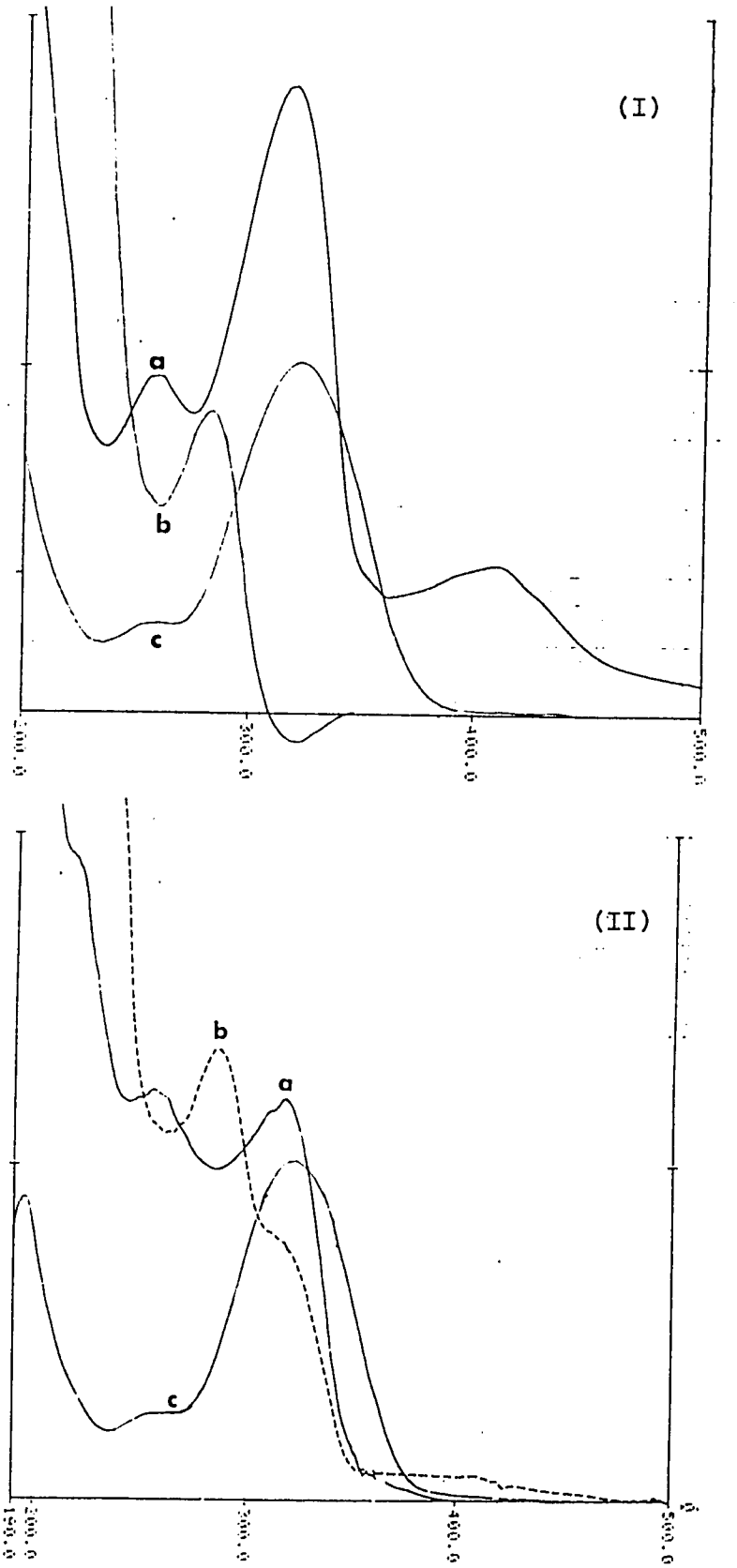


Figure 4. UV-spectrum of succinyl bruceine A-protein conjugates, BSA conjugate (I) and KHLH conjugate (II). BruceineA-BSA and BruceineA-KHLH conjugate (a);BSA and KHLH (b) and Free Bruceine A (c).

BEST AVAILABLE COPY

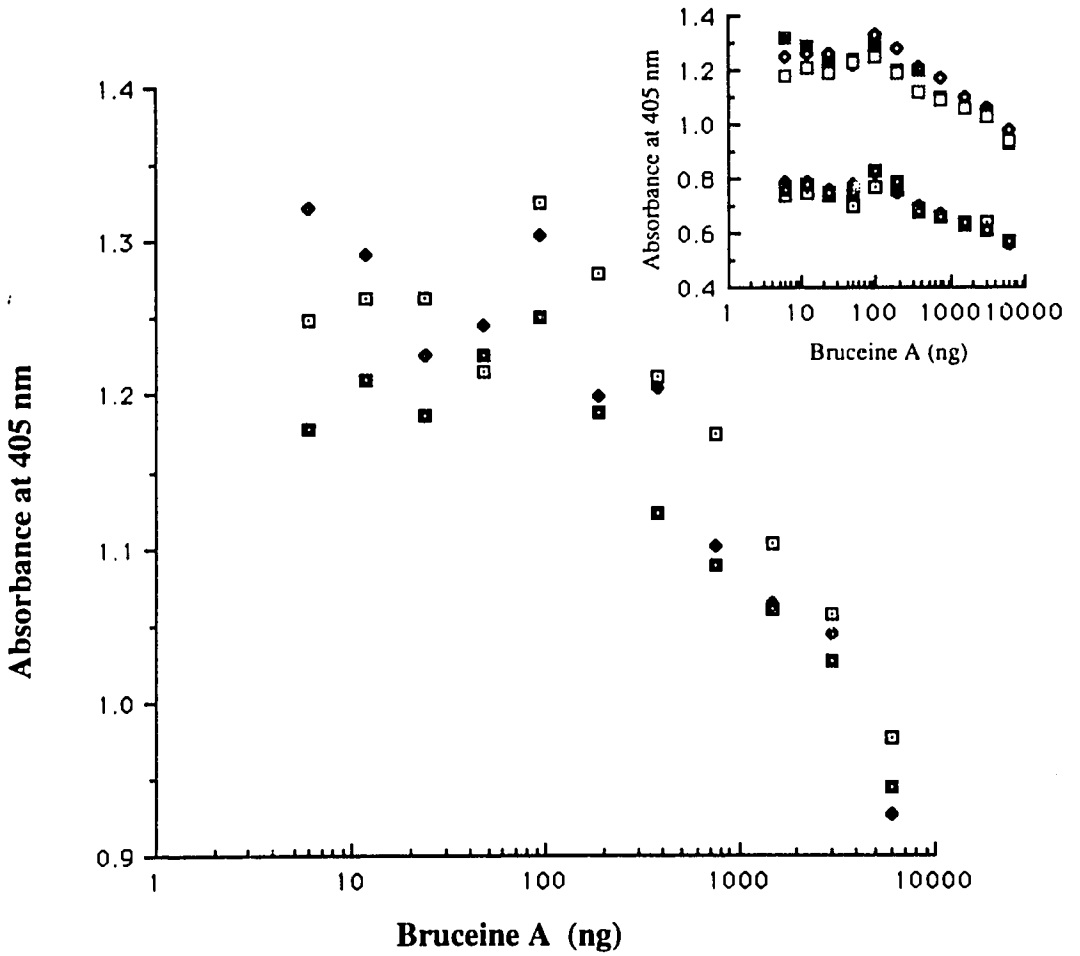


Figure 5. Standard curves for the bruceine A enzyme-linked immunoassay with antiserum (1/200dilution) as primary antibody (insert showing 1/200 ,upper, and 1/400, lower, dilution)

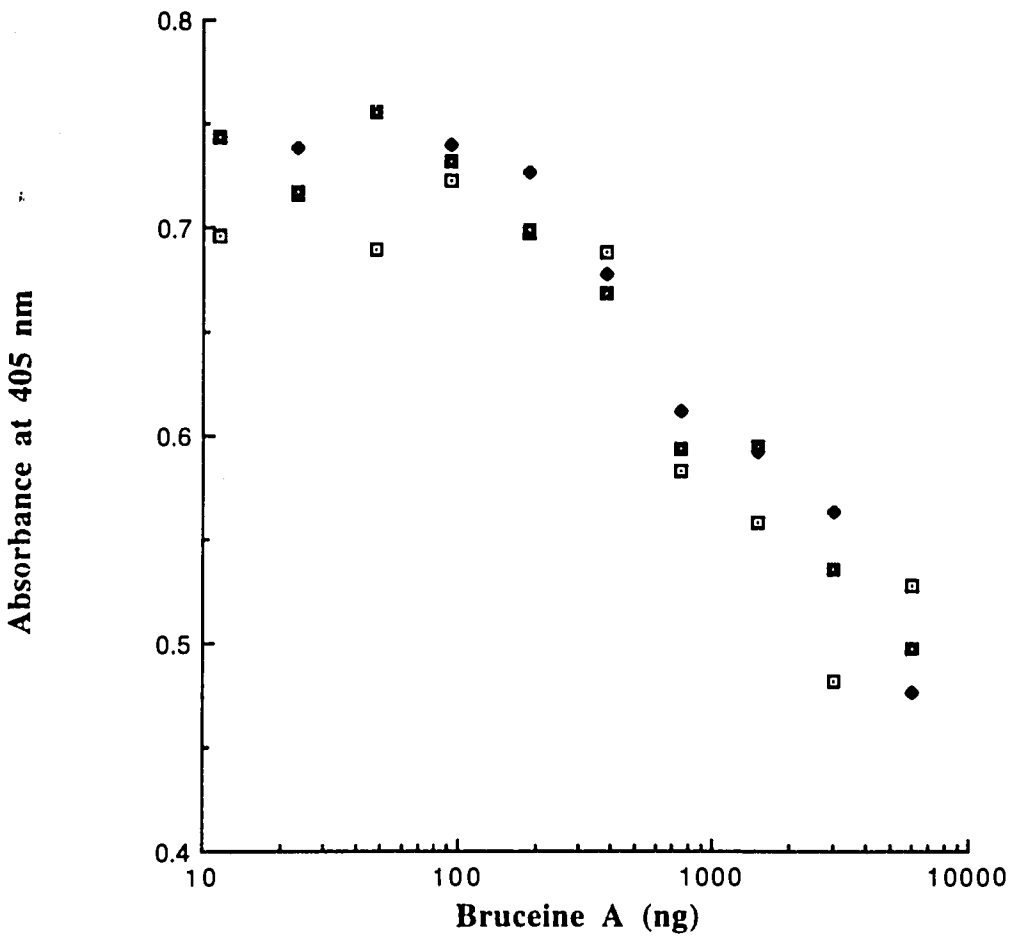


Figure 6. Standard curves for the bruceine A enzyme-linked immunoassay with specific IgG (1 μg) as primary antibody.

BEST AVAILABLE COPY

Impact, Relevance and Technology Transfer

The methodology of isolation and purification of antimalarial compounds from medicinal plants has developed. Therefore, the possibility to screening other plants apart from *Brucea javanica* has started. This will open up a new or more active compounds for antimalarial activity. The development of enzyme-linked immunoassay (EIA) for bruceine A will speeding up the selection program for improve high-yield bruceine cell clones from cell culture . The propagation of high yield plant will help a lot for developing country which use traditional medicine in helth problem.

This project has increase our capacity to be able to develope the EIA for detection of hepten (low molecular weight compound). This allowed us to extented EIA for other compounds which is important in food industry as a quality control apart from analyse plant constituents.

Project Activities/Outputs

Publication on " The Development of EIA against Bruceine A" is in preparation.

Project Productivity

Only part of the proposed goals of the project is accomplished, because the extraction of *B. javanica* yielded only bruceine A as an active quassinoid. Hence EIA for bruceine A was developed. Cross-reactivity has not tested due to lack of pure compounds which can not obtained from other laboratory in sufficient amount.

Future Work

The potential future developments in the area of this project which we foresee are :

1. Plants used for treatment of malaria need to be investigated scientifically in order to assess their potential as sources of new drug molecules.
2. Chemical modification of antimalarial active molecules to enhance their activities are possible.
3. Synthesis of compounds of known activity need to be studied.
4. Whenever a rare compounds interested is not available in pure form, or in insignificant quantities, the production of monoclonal antibodies (McAb) for a group selective antisera will be a method of choice.
5. The combination method of fast separation techniques such as HPLC, with group selective antisera produce by McAb technique will facilitate the analysis of complex mixtures of plant extract.

Literature Cited

Arisawa,M.,Handa,S.S.,MePherson,D.D.,Lankin,D.C.,Cordell,G.A., Fong,H.H.S. and Farnsworth,N.R. (1984) *J. Nat .Prod.* 47, 300.

Arnoldi,A., Arnone,A. and Merlini,L. (1984) *Heterocycles* 22, 1537.

Gillin,F.D. Reiner,D.S. and Suffness,M.(1982)*Antimicrob. Agents Chemother.* 22,342.

Guru,P.Y. Warhurst,D.C., Harris,A., Phillipson,J.D. (1983) *Ann. Trop. Med. Parasit.* 77, 433.

Keene,A.T., Harris,A., Phillipson,J.D. and Warhurst,D.C. (1986) *Planta Med.* 52 , 278.

Key,J.D. "Chinese Herbs, Their Botany, Chemistry and Pharmaco dynamics", Charles E. Tuttle Co., Rutland,VT, 1976

Lee,K.H., Hayashi,N., Okano,M., Nazaki,H. and Juichi,M. (1984) *J. Nat. Prod.* 47, 550.

Long-ze,L., Jim-Sheng,Z., Zhong-Liang,C., Ren-Sheng,X. (1982) *Acta Chimica Sinica* 40, 73.

Meisner,H. and Meisner,P. (1981) *Arch. Biochem. Biophys.* 208, 146.

O'Neill,M.J., Bray,D.H., Boardman,P., Phillipson,J.D., Warhurst,D.C., Peter,W. and Suffness,M. (1986) *Antimicrob. Agents Chemother.* 30, 101.

O'Neill,M.J., Bray,D.H., Boardman,P., Chan,K.L. and Phillipson,J.D. (1987) *J. Nat. Prod.* 50, 41.

Pavannard,K., Natukul,W., Dechatiwongse,T., Yoshihira,K., Yongva

nitchit,K., Scovill,J.P., Flippen-Anderson,J.L., Gilardi,R., George,C., Kanchanapee, P. and Webster,H.K. (1986) *Planta Med* . 52, 108.

Phillipson,J.D. and Darwish,F.A. (1979) *Planta Med.* 35, 308.

Phillipson,J.D. and Darwish,F.A. (1981) *Planta Med.* 41, 209.

Pierre,A., Gero,M.R., Tempete,C., Polonsky,J.(1980)*Biochem.Biophys. Res. Comm.* 93, 675.

Ray,A.B., Chatlopadhyay,S.K., Konno,C. and Hikino,H. (1982) *Hetero cycles* 19, 19.

Tanaka,H., Kato,I. and Ito,K. (1985) *Heterocycles* 23, 1991.

Trager,W., Polonsky,J.(1981) *Am. J. Trop. Med Hyg.* 30, 531.

Wellems,T.E.(1991) *Parasitology Today* 7, 110.

Werndorfer,W.H., Kousnetov,R.L. (1980) *Bull. WHO* 58, 341.

The Development of EIA against Bruceine A

P. Chaimanee and N. Purkkao
Department of chemistry, Faculty of Science, Silpakorn University, Nakorn Pathom 73000, Thailand.

A quassinoid compound, bruceine A, was isolated from *Brucea javanica* (L) Merr. (Simaroubaceae) (1). The antimalarial activity of bruceine A was tested against *Plasmodium falciparum* (K-1, chloroquine resistance) *in vitro*. The growth was significantly inhibited after incubation in bruceine A and revealed an ID 50 value of 19 ng with the hypoxanthine method. For the work with cell culture of *Brucea javanica*, a sensitive method for detection of bruceine A was required. An EIA would enhance the selectivity program.

Bruceine A has a relative molecular mass of 522 and could therefore not in itself raise an antibody response, so a hepten-carrier conjugate was made. The coupling of succinyl bruceine A to BSA was carried out using carbodiimide as reagent. A bruceine A-KHLH conjugate to be use as a coating conjugatein the EIA was synthesized in the same way.

The bruceineA-BSA conjugate was used to immunized rabbits. The obtained antiserum was tested in an indirect EIA. The measuring range extends from 50 to 6000 ng.

Executive Summary

Research objectives

Isolation of potential antimalarial compounds, bruceolides, from *Brucea javanica* (L) Merr. fruit. Development of enzyme-linked immunoassay (EIA) for the determination of bruceolide compounds isolated.

Research accomplishment

Two antimalarial compounds, bruceine A and cleomiscosin A were isolated from *B. javanica*. EIA for bruceine A was developed with measuring range extent from 50 to 6000 ng bruceine A.

Scientific Impact of Collaboration

Collaborating with Prof. Y. Yuthavong at Mahidol University, Thailand for antimalarial activity test was established.

Collaborating with Prof. W.C. Taylor at Sydney University and Prof. J.B. Bremner at Wollongong University, Australia for structure elucidation was established.

Collaborating with Prof. E. Mansell at Maiami University, USA for development of EIA was not initiated since investigator can not travel to USA due to maternity leave during the project period.

Description of the project Impact

EIA for bruceine A developed will be further used in screening program in cell culture of *B. javanica*.

Strengthing of Developing Country Institution

The project enable the investigator to establish the research work on isolation and purification of antimalarial compounds from other plants. The development of EIA for other biological active compounds can be pursued. This also strengthing us to develope EIA for quality control of compounds in food industry.