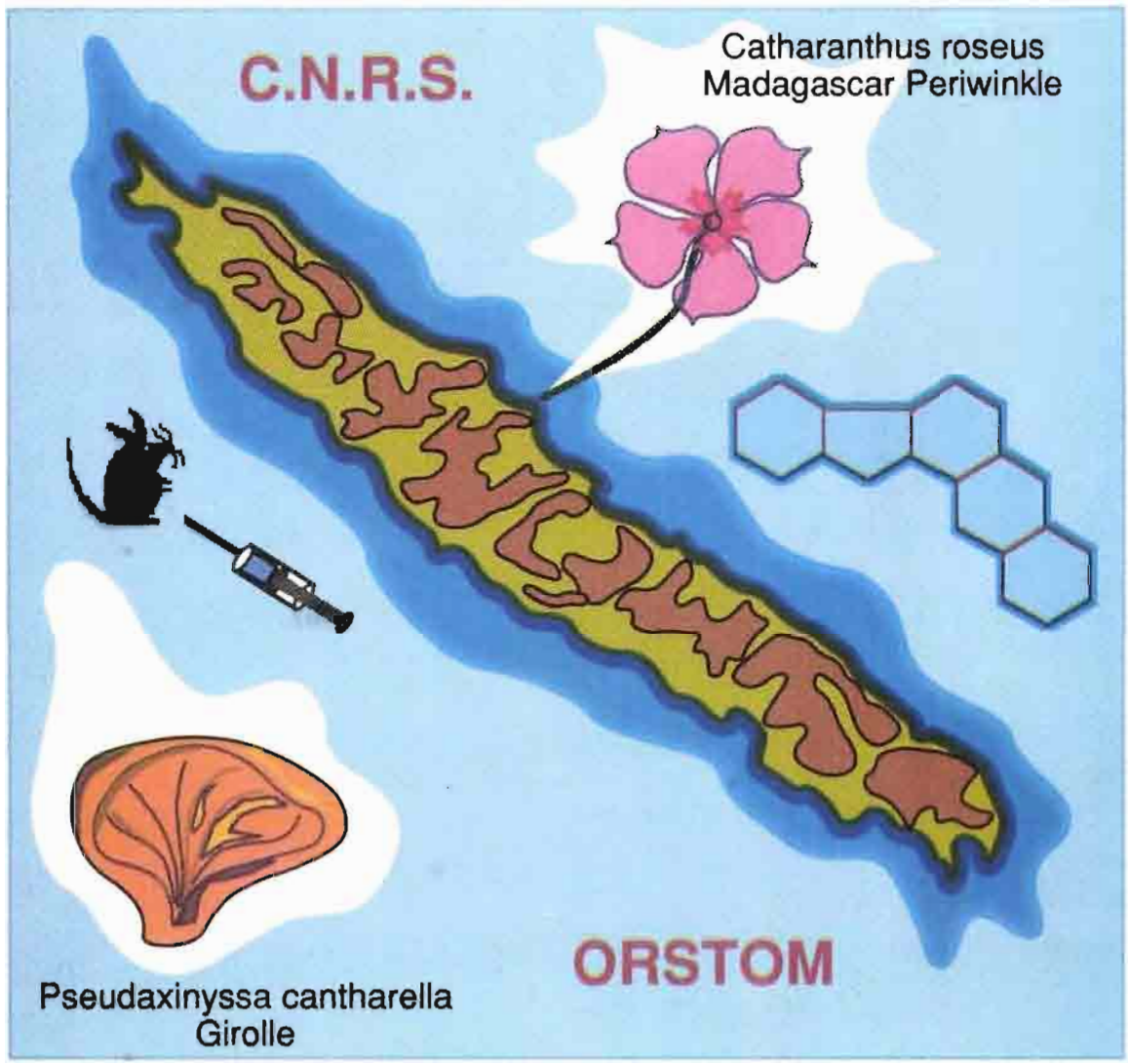
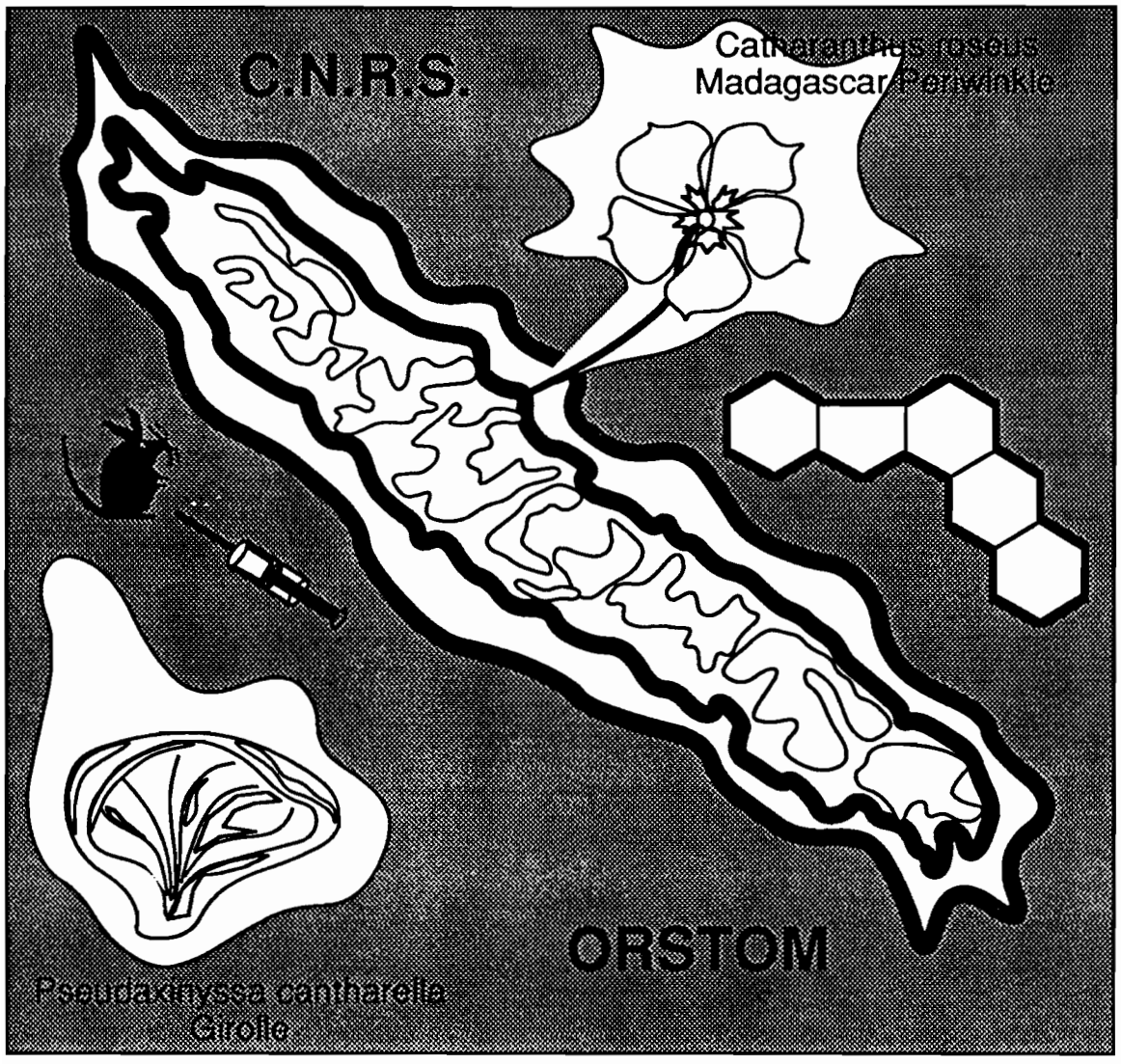


Troisième Symposium sur les substances naturelles d'interêt biologique de la région Pacifique-Asie

Third Pacific-Asia Symposium on biologically active natural products

Nouméa, Nouvelle-Calédonie, 26-30 Août 1991



Troisième Symposium sur les substances naturelles d'interêt biologique de la région Pacifique-Asie

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Le Comité d'Organisation tient à remercier tout particulièrement les Sociétés et les Organismes officiels suivants pour l'aide matérielle et le soutien financier indispensables à l'organisation de ce Symposium.

The Organizing Committee would like to warmly thank the following Companies and Official services for their help which were essential for the Symposium organization.

Centre National de la Recherche Scientifique (CNRS)
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LABORATOIRES PIERRE FABRE.

Un Partenaire Naturel.

pour le développement pharmaceutique
de Substances Naturelles Biologiquement Actives.

Les Substances Naturelles : le facteur de croissance de Pierre FABRE.

Le groupe Pierre FABRE a été créé en 1961, à l'occasion du lancement d'un médicament fabriqué à partir d'un extrait de *Ruscus aculeatus*. Depuis, le groupe connait une forte croissance, mêlant un savoir faire traditionnel sur les substances naturelles et le fort potentiel créatif de ses chercheurs, scientifiques de haut niveau.

Pierre FABRE est maintenant une firme de rang et de taille internationale, représentée dans plus de 75 pays.

Ses activités couvrent un large spectre, de la dermo-cosmétique aux médicaments les plus éthiques, en oncologie. Les substances naturelles sont impliquées dans la plupart des succès du groupe.

Un partenariat réussi entre la Recherche et l'Industrie.

La philosophie du groupe est de sélectionner des substances naturelles biologiquement actives (*Ruscus aculeatus, Serenoa repens, Catharanthus roseus*...) et d'en dériver des composés chimiques, avec une activité renforcée, de façon à créer et développer des médicaments originaux.

Ce long processus créatif requiert beaucoup de compétences... et de la chance. C'est pourquoi Pierre FABRE est impliqué dans plusieurs collaborations avec des laboratoires de recherche extérieurs.

L'une d'entre elles est particulièrement remarquable : L'Institut de Chimie des Substances Naturelles du C.N.R.S. (Gif-sur-Yvette) a mis au point un procédé de synthèse de la Vinorelbine, une molécule dérivée des alcaloïdes du *Catharanthus roseus*. Le C.N.R.S. et Pierre FABRE ont signé un accord de collaboration pour le développement de cette molécule en 1982. Ce projet a maintenant débouché sur le marché mondial avec la NAVELBINE qui a démontré son efficacité dans le cancer du poumon non à petites cellules et dans le cancer du sein.

Laboratoires Pierre FABRE - R&D coordination - 1, avenue d'Albi 81100 CASTRES - FRANCE
Tél - (33) 63 71 47 00

LABORATOIRES PIERRE FABRE.

A Natural Partner

for the pharmaceutical development
of Biologically Active Natural Products.

Natural Products : The growth factor of Pierre FABRE.

The Pierre FABRE Group was created in 1961, by launching a medicine coming from an extract of *Ruscus aculeatus*. Since then, the group has grown extensively, mixing a traditional know-how on natural products and the high creative potential of its top level scientists.

Pierre FABRE has now reached the rank and size of an international corporate group, represented in over 75 countries.

Its activities cover a wide range of products, from dermo-cosmetics to highly ethical medicines for oncology. Natural products are implied in most of the success-stories of the group.

A successfull partnership between research and industry.

The philosophy of the group is to select biologically active natural products (*Ruscus aculeatus, Serenoa repens, Catharanthus roseus*..) and to derive chemical compounds from them, with an enhanced activity, in order to create and develop original medicines.

This long creative process requires many skills, and... luck. That is why Pierre FABRE is involved in several collaborations with external research laboratories.

One of them is particularly successfull : The "**Institut de Chimie des Substances Naturelles**" from the C.N.R.S. (Gif-sur-Yvette) is the inventor of a synthesis of Vinorelbine, derived from the alkaloids of the *Catharanthus roseus*. The C.N.R.S. and Pierre FABRE have signed an agreement to collaborate for the development of this molecule in 1982. Now this project has reached the world market with NAVELBINE which has proved its efficacy on non small-cell lung cancer and breast cancer.

Laboratoires Pierre FABRE - R&D coordination - 1, Avenue d'Albi 81100 Castres - France.
Tel - (33) 63 71 47 00.

COMITÉS
COMMITTEES

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COMMITTEES

Le Symposium est placé sous le haut patronage de Monsieur Hubert CURIEN, Ministre de la Recherche et de la Technologie

The Symposium is held under the auspices of Mr Hubert Curien, Minister of Research and Technology.

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INFORMATIONS GÉNÉRALES
GENERAL INFORMATIONS

INFORMATIONS GÉNÉRALES
GENERAL INFORMATIONS

Secrétariat : le secrétariat est assuré par M^me^ Michèle DUBOIS et M^elle^ Kathia INTRANOVA.
Secretariat : *the secretariat is held by Mrs Michèle DUBOIS and Miss Kathia INTRANOVA*

Langue officielle : le français et l'anglais sont les langues officielles du Congrès.
Languages : *french and english are the official languages of the Meeting.*

Reconfirmation des billets d'avion : les reconfirmations seront faites par le secrétariat. Les participants sont invités à déposer la fiche "reconfirmation des vols" lundi 24 août au matin.
Flight confirmation : *the flight confirmations will be done by the secretariat. The participants are kindly requested to fill the "flight confirmation" form and give it to the secretariat on Monday morning, August 24th.*

Monnaie : la monnaie officielle est le Franc Pacifique (F CFP) : 100 F CFP = 5,50 FF ≈ 0, 90 US$. Deux agences de la Banque de Nouvelle Calédonie "Société Générale" et "Banque Nationale de Paris" sont situées à proximité des Hôtels sur l'Anse Vata. Ces agences sont ouvertes du lundi au jeudi de 7h30 à 16h00 et le vendredi de 7h30 à 15h00. Il n'y a pas de Banque ouverte le week-end. Il est possible de changer les F CFP à l'aéroport dans la salle de départ.
Currency : *the new caledonian currency is the Pacific Franc (F CFP) : 100F CFP=5,50FF ≈ 0,90 US $. There are two agencies of the New Caledonian Bank, "Société Générale" and "Banque Nationale de Paris" close to the Hotels on Anse Vata. The opening hours are 7h30 until 16h00 from Monday to Thursday and 7h30 until 15h00 on Friday. There is also a currency exchange desk in the departure loundge of the airport for exchanging the local money left at the end of your stay.*

Hébergement et repas : tous les participants sont logés dans les hôtels de la baie de l'Anse Vata. Les déjeuners peuvent être pris dans les nombreux cafés et restaurants proches du centre ORSTOM et des hôtels. Les cocktails, les repas du mercredi soir au KUENDU Beach et des excursions, sont compris dans le montant des droits d'inscription.
Meals and Housing *: most of the participants are staying in the hotels at the Anse Vata bay. There is a great variety of restaurants and coffee shops close to the ORSTOM centre and the hotels at all prices. Cocktails and the meals of Wednesday evening (KUENDU beach) and of field trips are included in the registration fee.*

Téléphone : un téléphone international à carte est disponible sur la promenade de la plage, à 100m du centre ORSTOM. Les cartes seront vendues par le Service des Postes. Elles sont aussi en vente dans les hôtels et les curios.

Phone : *an international call box is available on the Beach walk (100m of the ORSTOM Centre). It works with special cards that will be on sale at the Post Office desk. These cards are also available in Hotels and souvenirs shops.*

Philatélie : le Service des Postes et Télécommunications émet un timbre commémoratif du Congrès et l'enveloppe Premier Jour sera en vente au début du Congrès.

Philately : *the Post and Phone Office is publishing a stamp for the meeting; the first day envelope will be for sale on the second day of the meeting.*

Energie électrique : la Nouvelle-Calédonie est alimentée en 220 Volts, 50 périodes.

Electricity : *in New Caledonia electric current is 50 Hz , 200 V AC.*

Agence de voyages : plusieurs agences de voyages à l'Anse Vata près des hôtels peuvent se charger de réservations sur des vols intérieurs et sorties touristiques.

Travel agencies : *several travel agencies are located on the Anse Vata close to the hotels for domestic flights and touristic trips.*

Programme d'occupation des loisirs :

Un cocktail d'accueil offert par Monsieur le Maire de Nouméa aura lieu dans le Hall de l'ORSTOM le lundi 26 août au soir.

Le dîner de gala aura lieu le mercredi 28 août au restaurant Kuendu Beach. Départ en bus du centre ORSTOM à 19h00 précises.

Un cocktail de clôture sera offert avec la participation de la Société LV-MH le vendredi 30 août.

Les participants peuvent visiter le Parc Forestier et l'Aquarium de Nouméa (tickets dans la pochette).

Social events: *A welcome cocktail is offered by the Mayor of Nouméa, in the main Hall of ORSTOM centre on Monday evening, August 26th.*

The meeting dinner will be held at the Kuendu Beach restaurant. Departure from ORSTOM centre at 19h00.

The final cocktail is offered by the Meeting and the LV-MH industries in the main Hall of ORSTOM centre on Friday evening, August 30th.

Participants may visit the "Parc Forestier" (botanical garden) and the Aquarium of Noumea (tickets in the meeting-bag)

REMARQUES PRELIMINAIRES
PRELIMINARY REMARKS

Remarques préliminaires
Preliminary remarks

Traduction simultanée : la traduction simultanée en anglais des conférences et communications effectuées en français sera assurée. Pour faciliter le travail des interprètes, les conférenciers et présentateurs de communications orales sont priés d'apporter une copie de leurs textes au Secrétariat la veille de leur communication.

***Simultaneous translation** : the conferences and communications in French will be translated into English. To facilitate simultaneous translation, speakers are required to bring their texts to the Secretariat the day before the session.*

Diapositives : les diapositives doivent être déposées au secrétariat avant chaque session.

***Slides** : slides must be given to the Secretariat before the beginning of each session*

Rétroprojecteur : un rétroprojecteur est à la disposition des conférenciers.

Overhead projector : an overhead projector is available for speakers.

Publication des communications : les textes complets seront publiés par les éditions de l'ORSTOM durant le premier trimestre 1992. Les disquettes et textes imprimés complets avec formules, schémas et photos doivent être déposés au Secrétariat du congrès. Les textes manquants seront reçus au centre ORSTOM de Nouméa jusqu'au 15 octobre dernière limite. Les actes seront expédiés gratuitement aux participants dès leur parution.

***Publication of full papers** : the full papers will be published by ORSTOM editors during the first trimester of 1992. The disks and printed full papers (texts, formulas, schemes and pictures) should be given to the Secretariat. The missing texts would be sent to the ORSTOM Centre in Noumea before the 15th of October (deadline). The Proceedings will be sent free to participants as soon as they are published.*

Excursions scientifiques :

Samedi 31 août : une excursion en mer au Phare Amédée avec déjeuner sur l'îlot donnera un aperçu des fonds marins et du récif barrière. Des baptêmes de plongée, des promenades en apnée et des plongées pour plongeurs expérimentés sont possibles : les intéressés sont priés de remplir la fiche "plongée" et la remettre lundi 24 août au matin au secrétariat du congrès.

Dimanche 1er septembre : excursion dans les terrains miniers du Sud (Chutes de la Madeleine, Col de Yaté). Aperçu de la flore endémique de Nouvelle-Calédonie.

***Field trips** :*

Saturday, August 31st : a trip in the lagoon and to the Barrier reef with a lunch on the Amédée Islet should give to the participants a good look of the coral reef and lagoon fauna. There is the opportunity for beginners to do a shallow water SCUBA dive, as well as for experienced divers a trip on the outer reef (weather permitting). Snorkling equipement is available on the islet. People interested in diving or snorkling should fill the "Diving" form and give it to the secretariat on Monday, August 24th.

Sunday, September 1st : drive to the mining area in the South (Madeleine Waterfalls, Yaté lookout point) and have a look on the endemic flora of New Caledonia.

PROGRAMME SCIENTIFIQUE ET RÉSUMÉS DES COMMUNICATIONS

SCIENTIFIC PROGRAM AND ABSTRACTS OF COMMUNICATIONS

Vendredi 23 Août
Friday, August 23

13h00-16h00 : Inscription des participants arrivés ce jour. Les participants arrivant sur d'autres vols seront inscrits à leur arrivée.
Registration of participants arrived on Friday's flights. Particpants arriving on other flights will be registered at their arrival.

Lundi 26 Août
Monday, August 26

8h30-9h30 : Ouverture officielle
Official opening

- Jean FAGES — Directeur du Centre ORSTOM de Nouméa
Manager of the ORSTOM Center in Noumea

- Alain CHRISTNACHT — Délégué du Gouvernement en Nouvelle Calédonie; Haut - Commissaire de la République
High-Commissioner of New Caledonia

- Christian HABAULT — Délégué régional à la Recherche
Research and Technology Office representative

- Bernard PHILIPPON — Directeur du Département Santé de l'ORSTOM, Président du Congrès
Head of the Health Department (ORSTOM), Chairman of the Meeting

- Pierre POTIER — Directeur de l'Institut de Chimie des Substances Naturelles du CNRS, Président du Congrès
Head of the Natural Products Chemistry Research Institute (CNRS), Chairman of the Meeting

Session du matin
Morning session

Chairman : Pr John D. PHILLIPSON

9h30-10h30: Conférence Plénière, *Plenary Lecture*
Peter G. WATERMAN
"Strategies in the search for novel bioactive secondary metabolites" ...p.98

10h30-11h00: Pause Café - *Coffee Break*

11h-11h20 : Nordin Hj. LAJIS
"Chemical and some pharmacological studies of Malaysian medicinal plants"...p.61

11h20-11h40: Duangta KANJANAPOTHI, A. PANTHONG, T. TAESOTIKUL ...p.59
"Pharmacological activities of some plants of family Zingiberaceae"

11h40-12h10: Daniel GUÉNARD,
"Tubuline-microtubules systems and the research of new antimitotic drugs" ...p.51

Lundi 26 Août
Monday, August 26

Session de l'après-midi
Afternoon session

Chairman : Pr John D. FAULKNER

15h30-16h30 : Conférence plénière, *Plenary lecture*
M.VALERIA D'AURIA, F. DE RICCARDIS, L. GOMEZ PALOMA, M. IORIZZI, R. RICCIO, Luigi MINALE ...p.71
"Marine Natural products : chemical constituents from New Caledonian deep-water species"

16h30-16h50 : J.RODRIGUEZ, Ricardo RIGUERA, C. DEBITUS ...p.86
"Cytotoxic bis-pyrones from *Onchidium* sp. : absolute stereochemistry of onchitriols I and II"

16h50-17h10: Elly KOURANY-LEFOLL, T. SÉVENET, D. GUÉNARD, A. MONTAGNAC, C. DEBITUS, E. GUITTET, M. PAIS ...p.60
"Phloeodictine and thiophloeodictine, novel antimicrobial and cytotoxic guanidine alkaloids from the New Caledonian sponge : *Phloeodictyon* sp."

17h10-17h30: Michèle GUYOT, M. LITAUDON, F. FRAPPIER, F. TRIGALO, M.T. MARTIN, F .MONNIOT ...p.52
"Lissoclinotoxins, antibiotic 1,2,3- trithiane derivatives : structure and synthesis"

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18h00-18h30 H. HE, Y. VENKATESWARLU, D. John FAULKNER, J.L. RIOS STEINER, E. CORCORAN, J .CLARDY ...p.46
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"Coral reef ascidians of New Caledonia"

19h00 - 20h30 : Cocktail d'accueil de M. le Maire de Nouméa au centre ORSTOM, hall d'entrée
Welcome cocktail from the Mayor of Nouméa in the main Hall of the ORSTOM Centre

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Morning session

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"Possibilités d'utilisation de substances lichéniques comme protecteurs solaires"

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"Total synthesis of 19-hydroxytubotaiwine. Assignment of absolute configuration to a natural isomer"

10h10-10h30: Dayar ARBAIN ...p.24
"Chemical study of some Sumatran plants"

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"Sapogenins and saponins from two *Albizia* species from New Caledonia"

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"Ardisiacrispin B, a prostaglandin like effect saponin from *Ardisia crispa* "

11h40-12h10: Rosalinda SOLEVILLA ...p.93
"Biological studies of the seeds of *Ipomea muricata* (Jacq.) Linné (Convolvulaceae)"

Session de l'après-midi
Afternoon session

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P2 Bernard BODO, A. JÖSSANG, P. JÖSSANG, H.A. HADI, T. SÉVENET ...p.27
Alkaloids from *Horsfieldia superba.*

P3 Luc-Olivier BRUN, C. MARCILLAUD, C. DEBITUS, D. DUHET ...p.30
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P4 Luc-Olivier BRUN, R. URBAIN, E. WACAPO, C. DEBITUS ...p.31
"A method for the evaluation of marine extracts toxicity for the coffee berry borer: *Hypothenemus hampei*"

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"Synthèse de peptides à activité cytolytique."

P22 J. ROVIROSA, Y. PALACIOS, E. QUEZADA, J. DARIAS, Aurelio SAN MARTIN...p.87
"Chilean marine organisms: secondary metabolites and its biological activities"

P23 Dominique SARAZIN ...p.88
"Structural characterization of modified starch."

P24 Michel SAUVAIN, C. MORETTI, V. MUÑOZ, C. DEBITUS, C. JARDEL, P. TIMON-DAVID, M. GASQUET ...p.89
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P30 G. DEFIEUX, Joseph VERCAUTEREN, T. SÉVENET ...p.97
"Isolation and structural elucidation of the major component from *Pleiocarpidia* sp.: 3-epi-19-S-dihydrocadambine."

Chairman : Pr Luigi MINALE

17h00 - 18h00 Conférence plénière, *plenary lecture*
John C. COLL, M. MAIDA, P.W. SAMMARCO ...p.36
"Marine chemical ecology : chemicals speak softly in all languages"

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Mercredi 28 Août
Wednesday, August 28

Session du matin
Morning session

Chairman : Dr Yves MAGNIER

8h30-9h30	Conférence plénière, *Plenary lecture* Anne Marie LEGRAND, S. PAUILLAC, M. CHINAIN ...p.65 "La Ciguatera en Polynésie Française"
9h30-9h50	Raymond BAGNIS, A. SPIEGEL, L. N'GUYEN, R. PLICHART "30 ans de surveillance sanitaire et épidémiologique de la Ciguatera à Tahiti"
9h50-10h10	Serge PAUILLAC, H. LABROUSSE, C. JEHL-MARTINEZ, A.- M. LEGRAND, S. AVRAMEAS ...p.77 "Problèmes posées par la détection de la ciguatoxine"
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Jeudi 29 Août
Thursday, August 29

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Morning session

Chairman : Dr André MENEZ

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10h00-10h30	Lourdes J. CRUZ "Phylogenetic specificity of *Conus* neuropeptides"	...p.37
10h30-11h00	Pause Café, *coffee break*	
11h00-11h30	Bernard BODO , S. REBUFFAT, C. AUVIN-GUETTE, L. CONRAUX, I. VUIDEPOT, M. MASSIAS, Y .PRIGENT "Membrane active peptides from *Trichoderma* sp."	...p.26
11h30-12h00	P.V. MLADENOV, Christopher MURPHY "Autotomy promoting factor (APF) in south west Pacific sea stars"	...p.76

Session de l'après-midi
Afternoon session

Chairman : Dr Georges MASSIOT

15h30-16h30	Conférence plénière, *plenary lecture* John D.PHILLIPSON "Antiprotozoal agents from higher plants"	...p.80
16h30-17h30	Conférence plénière, *plenary lecture* Swee Hock GOH, E. SOEPADMO, S.L. OO, M.S. YADAV, M. ZAKARIA, W.H. WONG, T.S. KAM, A.H. HADI, C.H. CHAH, A.W. NORHANOM, J. MOK "Chemical and bioactive principles from selected Malaysian plants" Hamid A. HADI "Phytochemical survey of Malaysian plants"	...p.49 ...p.53
17h30-17h50	Pause Café, *Coffee Break*	
17h50-18h20	Byung Hoon HAN "Chemical and biochemical studies on the biologically active natural products in Korea"	...p.54
18h20-18h50	Dominique BOURRET "Clefs pour le traitement rationnel des données empiriques de la pharmacopée traditionnelle"	...p.28
18h50- 19h10	Banasri HAZRA, S. PAL, A. BANERJEE " Isolation and synthesis of biologically active naphtoquinone derivatives from a plant source"	...p.55

Vendredi 30 Août 1991
Friday, August 30

Session du matin
Morning session

Chairman : Pr Pierre POTIER

8h30-9h30 Conférence Plénière, *Plenary lecture*
Ali ALMOURABIT, A. AHOND, M. BEDOYA-ZURITA, R. HENG, R. MARQUES BRAGA, C. POUPAT, P. POTIER ...p.22
"La girolline : structure et synthèse"
François LAVELLE, A. CURAUDEAU, M. BAYSSAS, A. AHOND, C. POUPAT, J. PUSSET, D. LAURENT, P. POTIER ...p.64
"Girodazole (girolline) : from the lagoon of Noumea to cancer patients"

9h30-10h00 Pierre BRAQUET, F. CLOSTRE ...p.29
"Ginkgolides : chimie, biologie, pharmacologie et perspectives cliniques"

10h00-10h20 S. LAHLOU, G. PELLISSIER, Pierre DEMENGE ...p.39
"How to corroborate a spinal mechanism of action for a drug. Example of the hypotension induced by a dopamine receptor agonist"

10h20-10h40 Jean-Marie, GROGNET, M. ISTIN ...p.50
"Métabolisme et pharmacocinétique des alcaloïdes de l'ergot de seigle : apport de l'immunologie analytique"

10h40-11h00 Pause Café, *Coffee Break*

11h00-11h20 Caroline DJIAN-CAPORALINO ...p.42
"Mise au point sur les substances nématicides produites par des microorganismes et des végétaux supérieurs"

11h20-11h40 David J. DE VRIES ...p.40
"Application of radioreceptor binding analysis to the detection of marine natural products with therapeutic potential"

Session de l'après midi
Afternoon session

Chairman : Pr Shigetoh MIYACHI

15h30-16h30 Conférence plénière, *Plenary lecture*
William FENICAL, P.R. JENSEN, C. PATHIRANA, J.A. TRISCHMAN, D. TAPIOLAS ...p.47
"New antitumor antibiotics from marine microorganisms"

16h30-17h00 Wataru MIKI ...p.70
Quenchers against singlet oxygen in symbiotic bacteria of marine sponges

17h00-17h30 Pause Café-*Coffee Break*

17h30-18h00 Clôture officielle, *Official closing session*
Gilbert BALAVOINE, Christian MORETTI

18h00-20h00 Cocktail de clôture, Hall du Centre ORSTOM,
Closing Cocktail, Centre ORSTOM main Hall

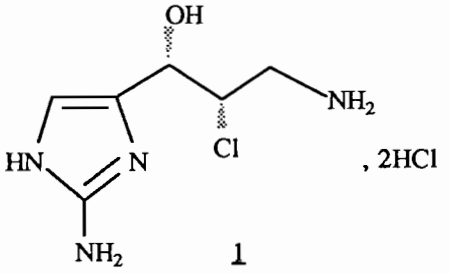
La girolline: structure et synthèse

Ali Almourabit, A. Ahond, M. Bedoya-Zurita, R. Heng, R. Marques Braga, C. Poupat et P. Potier

I.C.S.N./ C.N.R.S., 91198 Gif/Yvette Cédex, France

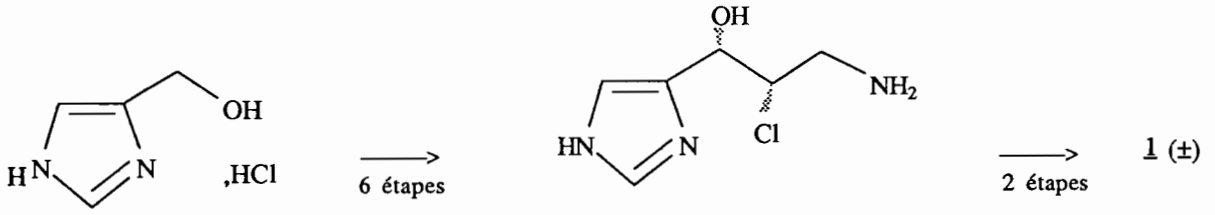
La girolline, 1, isolée d'une Eponge nouvelle du lagon néo-calédonien, *Pseudaxinyssa cantharella* C. LEVI, est un amino-2-imidazole porteur d'une chaîne polyfonctionnalisée : son activité antitumorale originale a suscité de nombreux travaux.

Sa structure a été déterminée essentiellement par l'utilisation des moyens spectroscopiques; sa stéréochimie a été précisée par synthèse, appuyée sur des résultats biologiques et confirmée récemment par analyse des spectres de diffraction des RX.

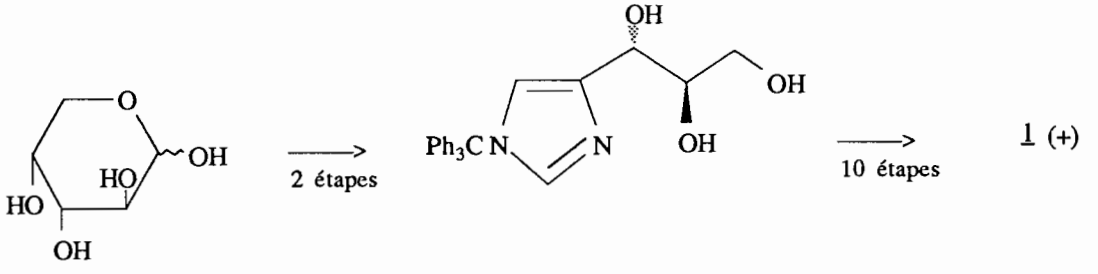


1

La première synthèse totale diastéréosélective a été réussie à partir de l'hydroxyméthyl imidazole.



Après de nombreux essais souvent infructueux de séparation des énantiomères et de synthèses énantiosélectives, une nouvelle synthèse stéréosélective vient d'être réalisée à partir du D-arabinose.



Ciguatera et remèdes traditionnels

Philippe Amade et D. Laurent

Centre ORSTOM, BPA5, Nouméa, Nouvelle Calédonie

Une liste de plantes utilisées dans la médecine traditionnelle pour le traitement de la ciguatera a été établie sur la base des études ethnobotaniques réalisées par l'Orstom dans les années 1980 au Vanuatu et en Nouvelle Calédonie, et de quelques données récentes (Remèdes traditionnels utilisés dans le Pacifique ouest contre la ciguatera, Bourdy, Cabalion, Amade, Laurent, à paraître).

Afin d'identifier et d'évaluer l'activité de ces remèdes traditionnels, nous avons mis au point un test sur souris qui consiste à traiter ces animaux, préalablement intoxiqués par de la ciguatoxine (extraite de foies de murènes), et à étudier leurs courbes pondérales.

La méthodologie utilisée et les premiers résultats obtenus seront discutés ici.

Le but de ce programme, après avoir sélectionné les remèdes actifs et testé leur éventuelle toxicité, est d'isoler et identifier le ou les principes actifs de ces préparations.

Chemical study of some Sumatran plants

Dayar Arbain

Department of Pharmacy, FMIPA, University of Andalas, Padang, West Sumatra, Indonesia

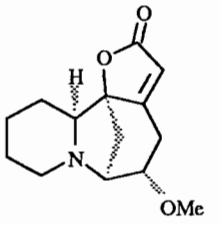
In our study of the chemistry of Sumatran plants, 908 species have been collected and tested for the alkaloids in the field using the Culvenor-Fitzgerald method.

Some alkaloids bearing plants having traditional medicinal value have been selected for further study and they have yielded known and new alkaloids.

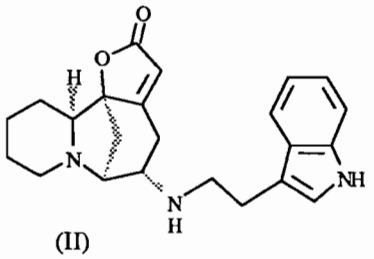
One of these plants, *Margaritaria indica* (Euphorbiaceae) was found to contain known alkaloids phyllochrysine, securinine, securitinine, securinol A and phyllanthine, together with new bases 15α-methoxydihydrophyllochrysine (I), margaritarine (II) and epiphyllanthine (III). The structure of securinol A has been revised to (IV).

The leaves of another species *Rauvolfia sumatrana* (Apocynaceae) have given a high yield of the new base methyl 12-hydroxyakummilan-17-carboxylate (V).

The isolation and chemistry of these compounds will be discussed.



(I)



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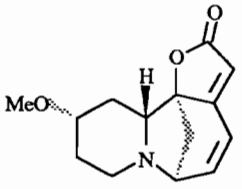

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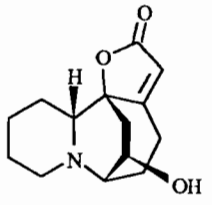

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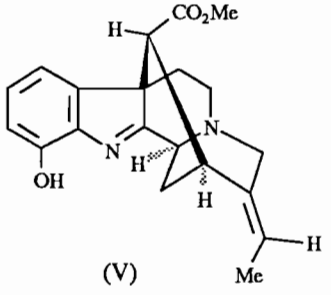


(V)

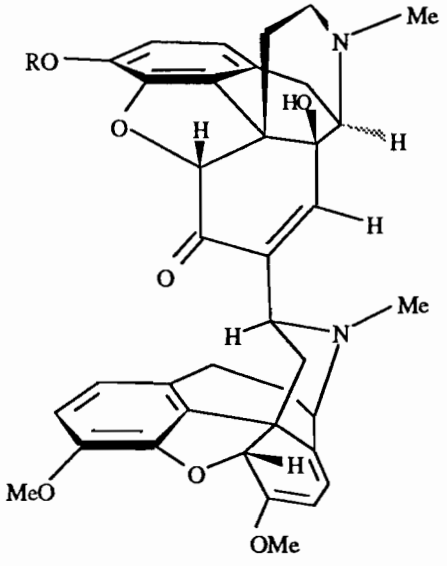
Dimeric alkaloids from Tasmanian-grown *Papaver somniferum*

C. Dragar and I. Ralph C. Bick

Chemistry department, University of Tasmania, Hobart, Tas., Australia

For the large-scale cultivation of opium poppy (*Papaver somniferum*) in Tasmania, a special variety has been developed to facilitate mechanical harvesting and to suit local conditions. While a high content of the most commercially valuable alkaloid, morphine, was maintained, considerable variation in the nature and content of the minor bases resulted, including the formation of hitherto unreported dimeric alkaloids.

From an alkaloidal extract of poppy heads, an amorphous alkaloid, $[\alpha]^{20}_{D}$ -297° ($CHCl_3$), was isolated and named somniferine (**1**). Its structural determination by spectroscopic means will be described, and that of another minor constituent, which proved to be O-methylsomniferine (**2**).



(**1**) Somniferine (R=H)
(**2**) O-methylsomniferine (R=Me)

Exhaustive extraction of poppy heads and straw yielded 1.88% of crude opium, from which the major alkaloids morphine (43.7%), thebaine (8.5%), codeine (4.6%), papaverine (3.4%), O-methylsomniferine (4.3%) and somniferine (1.9%) were isolated by a combination of column chromatography and the use of a chromatotron. The yield of the major components was confirmed by GC analysis, which also indicated the presence of oripavine in 0.3% yield. In addition to these constituents, a number of other alkaloids, including another dimeric base as well as monomeric components, were detected, amongst which palaudine, codeinone, laudanosine, and two isomers of thebaine N-oxide have so far been identified.

Membrane active peptides from *Trichoderma* species

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**Unité mixte de Recherche Bruker-CNRS, UMR 50, Université Louis Pasteur, 34 rue de l'Industrie, 67160 Wissembourg, France*

Trichoderma are widespread soil fungi and most of them exhibit antagonistic properties against other microorganisms, bacteria and fungi, due in part to antibiotic-antifungal compounds production. From several species and strains (*T. harzianum, T. longibrachiatum, T. saturnisporum, T. koningii*) of various geographical origin, we isolated antibiotic peptides : trichorzianins, tricholongins, trichogins, trikoningins, saturnisporins and harzianins. For each strain, peptides were obtained as microheterogenous mixtures of sequence homologues which were further separated by reversed phase HPLC.

The sequences were determined from FAB mass spectroscopy and high field ^{1}H and ^{13}C NMR. These hydrophobic peptides belong to the peptaibol class, as their N-terminal aminoacid is acetylates, their C-terminal residue is an amino alcohol and they have a high content in α-aminoisobutyric acid (Aib). They contain 11 to 20 residues and the N-terminal acyl group is either an acetyl (Ac) or an octanoyl (Oc) one, depending on the strain they originate from. As examples, the sequences of saturnisporin A IV (SA IV), harzianin A V (HA V) and trikoningin B 1 (KB I) are given below:

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*
SA IV:	Ac	Aib	Ala	Aib	Ala	Aib	Aib	Gln	Aib	Leu	Aib	Gly	Aib	Aib	Pro	Val	Aib	Iva	Gln	Gln	Pheol
HAV:	Ac	Aib	Gly	Ala	Aib	Iva	Gln	Aib	Val	Aib	Gly	Leu	Aib	Pro	Leu	Aib	Iva	Gln	Leuol		
KB I:	Oc	Aib	Gly	Val	Aib	Gly	Gly	Val	Aib	Gly	Ile	Leuol									

The conformation, either in solution in organic solvents or in presence of micelles, was shown to be mainly α-helical, from NMR and CD data.

Such peptides interact with biological membranes. Comparison of their membrane activity was studied by mesuring the peptide-induced leakage of a liposome-entrapped probe.

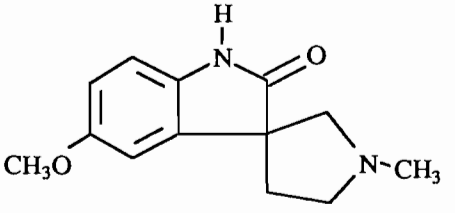
Alkaloids from *Horsfieldia superba*

Bernard Bodo, A. Jössang, P. Jössang, H.A. Hadi* and T. Sévenet**

Laboratoire de Chimie, Museum national d'Histoire naturelle, URA CNRS 401, 63 rue Buffon, 75005 Paris, France,
** Department of Chemistry, University of Malaya, Kuala Lumpur, 0102 , Malaysia,*
*** Institut de Chimie des Substances Naturelles, CNRS, 911198 Gif sur Yvette, France*

Several Myristicaceae are used as sources of intoxicating snuffs, and some of them have been shown to contain hallucinogenic alkaloids, especially those from *Virola* which contain tryptamine derivatives. Alkaloids were not previously reported in the *Horsfieldia* genus which encompasses several woody species growing in South East Asia and is sometimes used as a medicinal plant by the natives.

From *H. superba* , a small tree indigenous to Malaysia, were isolated from leaves three bases : horsfiline **1** which is a new oxindole alkaloid and the known 2-methyl-6-methoxy-1,2,3,4-tetrahydro-ß-carboline **2** and 5-methoxy-N,N-dimethyltryptamine.



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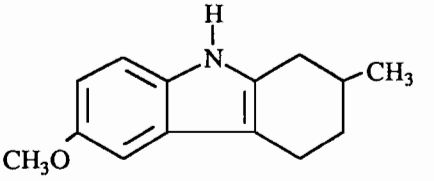

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The structure of **1** was determined by spectral analysis and confirmed by its partial synthesis from **2** by an oxidative rearrangement : the reaction of the tetrahydro-ß-carboline **2** with lead tetraacetate, furnished a 4a-acetoxyindolenine **3**, isolated by chromatography and characterized by spectral analysis. Compound **3**, was further converted by an acidic catalysed rearrangement into the oxindole (±) horsfiline, **4**, obtained as a racemic mixture. Identity of (-) natural **1** and (±) synthetic **4** horsfilines, except for the isomerization of the asymmetric center at C-3, arose from comparison of mass and NMR spectral data.

Clefs pour le traitement rationnel des données empiriques de la pharmacopée traditionnelle

Dominique Bourret

ORSTOM, 44, Rond Point du Jardin, 97400 Saint Denis de la Réunion, La Réunion

Le recueil des recettes végétales ou autres qui constituent les codex de la médecine empirique peut ressembler à un catalogue de pièces détachées ou d'antiquaire; c'est le reproche habituel que les pharmaco-chimistes font aux ethnobotanistes ou aux ethnologues qui, le plus souvent, produisent ces inventaires.

Deux philosophies semblent s'opposer, ici, celle des humanistes qui exaltent la tradition et celle des demiurges qui misent sur la science. En réalité, les deux écoles ne croient qu'en l'esprit humain. La première privilégie l'odorat et le goût, qui globalisent, la seconde la vue, qui dissèque. Le passage de l'une à l'autre s'est historiquement effectué avec le progrès des techniques et en concomitance avec la perte de la foi religieuse. L'art pictural en est le témoin.

Il s'agit donc, si l'on veut utiliser au profit de la science le patrimoine de connaissances accumulées par les civilisations, de réconcilier les sens et l'esprit. C'est, dans le contexte mélanésien ou nous nous situons, une lecture particulière du "DO KAMO" de Maurice Leenhardt.

Dans le cas des pharmacopées, si les approches anthropologiques classiques sous-estiment l'importance de la connaissance naturaliste dans la création socio-culturelle, les inventaires ou enquêtes ethnoscientifiques n'en doivent pas moins, pour être efficaces, être sélectifs : il ne s'agira plus d'établir un dictionnaire ou d'étudier l'environnement d'une société, mais de repérer les usages allant dans le sens de la recherche entreprise. Il convient de noter au passage qu'un tel repérage suppose malgré tout un certain acquis ethnologique. Les contre-sens dûs à l'ignorance des cultures abordées sont souvent à l'origine du mépris affiché pour les "croyances populaires". A cet égard, le traitement statistique, malgré son aspect réductionniste, offre des surprises.

En contre-partie, les expérimentateurs doivent mieux apprécier l'expérience accumulée par l'empirisme traditionnel. On peut remarquer, sur le sujet de l'éthique, une évolution récente de la doctrine. On n'exclue plus, sous garanties, l'expérimentation précoce sur l'homme. Cependant, en général, les modèles de laboratoire restent encore souvent des tamis trop préconçus, peut être parce qu'il est plus complexe d'analyser le fonctionnement d'un système que celui d'une cellule isolée, alors même que la tendance actuelle de la science est à l'étude globale de l'individu et de l'individu dans son milieu.

Il est indéniable que la question financière est prépondérante dans le choix expérimental; aussi les clefs proposées ci-dessus doivent-elles d'abord ouvrir des coffres forts.

Ginkgolides : chimie, biologie, pharmacologie et perspectives cliniques

Pierre Braquet et F. Clostre

Institut Henri Beaufour, 17 avenue Descartes, 92350 LE PLESSIS ROBINSON, FRANCE

La découverte de l'antagoniste spécifique du Ginkgolide B (BN 52021) vis-à-vis du "platelet activating factor" (PAF) montre qu'aujourd'hui comme hier, l'avenir de la chimie thérapeutique passe toujours - malgré les progrès de la synthèse organique - par l'exploration des substances naturelles.

Le Ginkgolide B est un des principes actifs d'un extrait standardisé de feuilles de *Ginkgo biloba* L. (GBE761). Cet extrait comprend deux principaux groupes de principes actifs:

- des flavonoïdes sous forme d'hétérosides de quercetine, de kaempférol et d'isorhamnétine
- des substances terpéniques spécifiques : les Ginkgolides et le bilobalide.

Parmi les Ginkgolides d'origine naturelle, c'est le Ginkgolide B qui s'est avéré le plus puissant antagoniste du PAF. Il présente une structure chimique en cage très originale, en C20, incorporant un groupe t-Butyle et 6 cycles à cinq chaînons dont un système spiro [4-4] nonane, un tétrahydrofurane et trois lactones. Sa synthèse totale a été réalisée par E.J. Corey, ainsi que celle d'analogues structuraux incluant des modifications diverses du squelette, ayant permis de dégager les relations structure-activité.

C'est en 1984 que l'antagonisme spécifique du Ginkgolide B vis-à-vis du PAF fut démontrée sur des plaquettes sanguines. Rappelons que le PAF trouve son origine dans les phospholipides des membranes de nombreuses cellules de l'organisme. Il est sécrété par les membranes cellulaires des plaquettes, des leucocytes (polynucléaires neutrophiles et basophiles), monocytes, macrophages (en particulier alvéolaires), mais aussi de cellules vasculaires comme les cellules endothéliales. Le PAF est un médiateur intercellulaire circulant que l'on peut apparenter, quant à sa signification physiologique générale, à d'autres autacoïdes comme l'histamine, les prostaglandines ou les leucotriènes. Mais, à l'encontre de ceux-ci, il n'est pas stocké dans des vésicules mais synthétisé et libéré extemporanément après sensibilisation et stimulation cellulaires.

Les implications du PAF en pathologie sont nombreuses et leur explorations doivent beaucoup à la découverte d'antagonistes de référence tels que le Ginkgolide B.

Les nombreux travaux biologiques et pharmacologiques réalisés dans le monde entier avec le BN 52021 (1, 2) ont permis de mettre en évidence un grand nombre de perspectives thérapeutiques dont certaines sont actuellement en phase III d'études cliniques. Les principales orientations sont : les ischémies tant cérébrales et cardiaques que périphériques et rénales, certains états de chocs, les rejets de greffes cardiaques et rénales et le traitement des maladies autoimmunes (e.g. sclérose en plaques) mais aussi les altérations de la muqueuse gastro-duodénale et les réactions d'hyperréactivité bronchique.

Références:

1 Ginkgolides: chemistry, biology, pharmacology and clinical perspectives (vol.1). P. Braquet Ed., 1988, J.R. Prous Barcelona Publisher

2. Ginkgolides: chemistry, biology, pharmacology and clinical perspectives (vol.2). P. Braquet Ed., 1989, J.R. Prous Barcelona Publisher

Acaricidal activity of marine organisms to the cattle tick : *Boophilus microplus*

Luc Olivier Brun, C. Marcillaud, C. Debitus et D. Duhet

Centre ORSTOM, BPA5, Nouméa, Nouvelle Calédonie

Boophilus microplus is the most important tick species in New Caledonia. It is responsible for physical damage to livestock, sometimes leading to paralysis or death of animals, it can also transmit various serious desease. In New Caledonia as in most countries *B. microplus* is control ed by regular dipping of cattle in insecticides. This species has demonstrated the widest spectrum of resistance to acaricides in all continents and in New Caledonia the first case of resistance was experienced in 1983 (BRUN *et al.*) following control failure.

As part of the SMIB programme (Substances Naturelles d'Intérêt Biologique) acaricidal activity of extracts made from 150 organismes was studied during the last three years. Samples were collected in the new Caledonian lagoon as well as in blue water, between 300 and 700 meters deep, mainly by draggings.

A simple method derived from Stone and Haydock (1962) was developed to screen biological activities of dried freeze organismes using larvae, the most susceptible cattle tick stage, for toxicity assessment.

Most of the active compounds were found among sponges and gorgonians.

Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

A method for the evaluation of marine extracts toxicity for the coffee berry borer : *Hypothenemus hampei*

Luc-Olivier Brun, R. Urbain, E. Wacapo et C. Debitus

ORSTOM, centre de Nouméa, BP A5, Nouméa, Nouvelle Calédonie

Hypothenemus hampei is the major pest of coffee worlwide. It is a pest exclusively of coffee berries. Apart from dispersive flight by adult females, all the life cycle of *H. hampei* is passed inside the coffee bean. Very few insecticides are available for the control of this pest due to the cryptic nature of its life cycle. High level of endosulfan (the most active synthetic pesticide) resistance in *H. hampei* was discovered in New Caledonia (Brun et Ruiz, 1987) following outbreak of the pest in all major coffee growing regions of the East Coast of the island. The recent development of an artificial media for rearing this pest has allow preliminary investigations of toxicity of extracts of marine organisms collected during SMIB program (Substances Marines d'intérêt Biologique) oceanographic campaigns.

Third Pacific-Asia Symposium on biologically active natural products, Noumea, New Caledonia, August 26-30th, 1991

Isolement et identification de deux saponines des feuilles de *Polyscias fruticosa* Harms var. feuilles jaunes (Araliacées)

A. Proliac, A. Chaboud, N. Gopalsamy *, J. Raynaud et Pierre Cabalion **

Laboratoire de Botanique, de Biologie Cellulaire et Pharmacognosie, Université Claude Bernard (Lyon I), France,
** Institut de Pharmacognosie et Phytochimie, Ecole de Pharmacie, Université de Lausanne, Suisse,*
*** ORSTOM, UR 4G (Substances Naturelles d'Intérêt Biologique), Département Santé, Paris, France*

Poursuivant nos recherches sur les saponines du genre *Polyscias*, nous avons étudié les composés de *Polyscias fruticosa* Harms var. feuilles jaunes, originaire de l'archipel de Vanuatu où elles sont utilisées en médecine populaire notamment comme anti-inflammatoire.

Les feuilles de cette plante se sont avérées riches en saponines triterpéniques. C'est ainsi que nous avons isolé les saponines I et II à l'état pur en combinant la chromatographie basse pression sur une colonne RP 18 suivie de filtration sur gel Séphadex LH 20.

Les structures de ces saponines ont été établies par des méthodes spectrométriques (de masse et de résonance magnétique nucléaire - RMN ^{1}H et RMN ^{13}C -) et par des méthodes d'hydrolyses (acide, alcaline et enzymatique).

Pour la saponine I (qui dérive de l'hédéragénine) nous retiendrons la structure suivante:

Rhamnose 1 —> 2 Arabinose 1 —> 3 (Hédéragénine)

Cette saponine est mentionnée pour la deuxième fois à l'état naturel; elle a été identifiée la première fois chez *Polyscias dichroostachya* Baker (1).

La saponine II a pour génine l'acide oléanolique. Nous avons établi pour elle la structure suivante:

Rhamnose 1 —> 4 acide glucuronique 1 —> 3 (Acide oléanolique) 28 <—1 Glucose

Ce composé rare correspond à l'olaxoside identifié pour la première fois dans le genre *Olax*.

L'extrait total des saponines de *Polyscias fruticosa* a montré une très forte activité molluscicide* vis à vis de *Biomphalaria glabrata*. Cette activité peut être rapportée à la saponine I qui est un monodesmoside dont l'activité molluscicide a été démontrée chez un autre *Polyscias* de l'Ile Maurice : *Polyscias dichroostachya* (1).

L'activité traditionnelle de *Polyscias fruticosa* peut se justifier par la présence de l'olaxoside qui possède des propriétés anti-inflammatoires (2).

* Nous remercions Monsieur le Professeur HOSTETTMANN, Directeur de l'Institut de Pharmacognosie et de Phytochimie, Ecole de Pharmacie, Université de Lausanne, qui a bien voulu nous accepter en stage dans son laboratoire et qui nous permis de réaliser cet essai.

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Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

Ethnopharmacologie des *Psychotria* de Vanuatu et essais biologiques des alcaloïdes isolés

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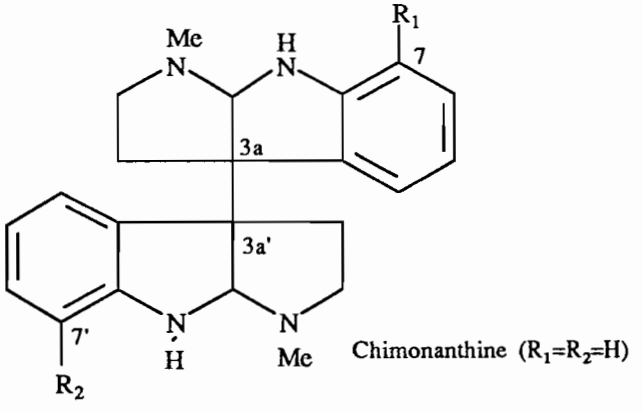
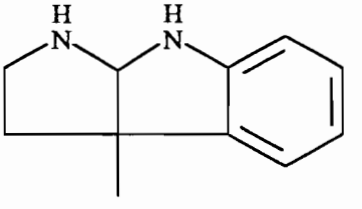
Le genre *Psychotria* (Rubiaceae) est représenté à Vanuatu par plusieurs espèces contenant des alcaloïdes et dont l'étude semblait justifiée par leur réputation en médecine vernaculaire.

L'étude botanique exhaustive du groupe taxonomique formé par les espèces du genre *Psychotria* de Vanuatu reste à faire. D'après nos relevés (exhaustifs pour les Herbiers de Port Vila, Nouméa et Paris), d'après la compilation probablement non exhaustive de la littérature et d'après notre connaissance du terrain, Vanuatu compterait **au moins 8 espèces** différentes de *Psychotria* (*P. trichostoma, P. aneityensis, P. forsteriana, P. milnei, P. nacdado*). Les trois autres espèces restent à définir botaniquement, mais aucune étude de fond n'est actuellement en cours sur la taxonomie de ce groupe.

P. trichostoma et *P. forsteriana* possèdent une composition chimique très voisine en alcaloïdes, *P. aneityensis* en semble dépourvu (sauf pour un seul spécimen), *P. nacdado* n'en contient pas, *P. milnei* semble correspondre pour le moins à **deux races chimiques** dont l'une dépourvue d'alcaloïdes. Les alcaloïdes isolés sont polyindoliniques, leur unité de base est la méthylpyrrolidinoindoline, les enchaînements se faisant par liaison 3a-3a' et 3a-7' (voir figures). Le grand nombre de carbones asymétriques entraîne l'existence de nombreuses isoméries, ce qui ne facilite pas les recherches, la stabilité des molécules étant de plus encore peu connue (étude RX difficile).



Chimonanthine (R_1=R_2=H)



pyrrolidinoindoline

Selon nos enquêtes ethnopharmacologiques, les indications médicinales des espèces de ce genre sont relativement rares. Dans le Nord de l'archipel, les feuilles de diverses espèces (*P.forsteriana, P. trichostoma* et *P.aneityensis*) entrent dans la composition de remèdes contre les **courbatures** et les **douleurs musculaires**. Par ailleurs, un auteur rapporte l'usage de *P. trichostoma* contre les **abcès**.

Les alcaloïdes totaux sont sédatifs du SNC chez la souris (test comportementaux) et cytotoxiques sur hépatomes de rat (HTC). Les polymères isolés sont actifs, sauf la calycanthine, sur le SNC de la souris, ils inhibent l'agrégation des plaquettes sanguines humaines (doses micromolaires), sont cytotoxiques sur les lignées HTC, Molt4 et Faza, et présentent une activité antibactérienne en particulier sur le staphylocoque doré.

Il est tentant de vouloir établir une corrélation entre les réputations locales des espèces médicinales et les activités pharmacologiques observées, par exemple dans le domaine de l'**inflammation** (études à poursuivre pour préciser le mode d'action) et de l'**activité antibactérienne** (éventuellement intéressante par voie externe).

Third Pacific-Asia Symposium on biologically active natural products, Noumea, New Caledonia, August 26-30th, 1991

Effet d'un saponoside tétrasaccharidique de *Mimosa pudica* L. sur les lymphomes humains et murins

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Originaire d'Amérique, le *Mimosa pudica* L. (Mimosaceae) a été acclimaté dans différentes régions tropicales. Il est utilisé en Chine pour traiter les affections des voies respiratoires et de l'appareil digestif (1), au Viêt-Nam comme sédatif et antirhumatismal (2) et comme antidysentérique, fébrifuge, diurétique, calmant dans les Caraïbes (3). A partir des graines de *M. pudica* , récoltées à Vanuatu, nous avons obtenu un saponoside tétrasaccharidique (MPS2) (4). Or, nous avons montré récemment que deux saponosides tétrasaccharidiques, isolés de *M. tenuiflora* (Willd.) Poiret possèdent des propriétés toxiques vis-à-vis de différents types de cellules animales en culture, alors que les hexa- et heptasaccharides correspondants n'ont aucune toxicité (5). Cette observation confirmait les résultats des travaux d'Anisimov (6) sur la toxicité cellulaire de saponosides tétrasaccharidiques isolés de *Holothuria std.*

Sur la base de ces résultats, nous avons testé l'activité de MPS 2, sur des cultures de cellules tumorales, afin de déterminer à la fois : * si une chaîne de quatre sucres conférait nécessairement à un saponoside une toxicité cellulaire, * si l'usage de cette plante en médecine traditionnelle ne devrait pas être reconsidéré, en cas de réponse positive.

L'activité de MPS 2 a été étudiée sur deux lignées tumorales leucémiques, l'une murine (RDM 4), l'autre humaine (Molt 4), dont la multiplication cellulaire s'effectue très régulièrement, à vitesse maximale très supérieure à celle des cellules normales. Ces modèles biologiques permettent donc de détecter non pas des effets stimulants sur la multiplication cellulaire, mais au contraire des activités inhibitrices ou cytotoxiques sur ce processus.

MPS 2 a été testé aux doses de $10^{-3}\mu M$, $5x10^{-4}\mu M$ et $10^{-4}\mu M$ sur les lignées Molt 4 et RDM 4 dont la viabilité cellulaire est évaluée à l'aide du test d'exclusion au Bleu Trypan et du test MTT, comparée à celle des cultures témoins.

Les résultats des deux tests d'évaluation concordent parfaitement. MPS 2 n'a montré ni activité inhibitrice de la croissance ni activité toxique sur ces deux lignées de cellules tumorales. Une chaîne tétrasaccharidique ne détermine donc pas nécessairement la toxicité d'un saponoside triterpénique.

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Lituarines, a new class of marine macrocyclic lactones isolated from the new caledonian sea pen *Lituaria australasiae*

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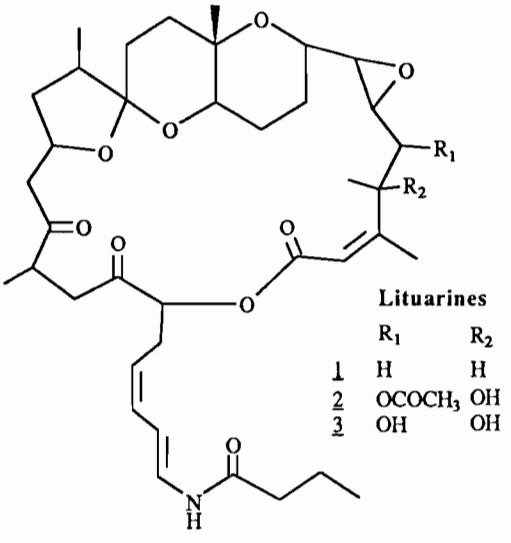
The coelenterates have been extensively investigated for biological activity, especially the order Alcyonacea (Soft corals) and Gorgonacea. Although many chemical studies on the Pennatulacea order (Sea pens) have been carried out, the Veretillidae family has been comparatively little investigated (1). The metabolites that have been isolated from Sea pens are all diterpenes (2).

In the course of our survey on physiologically active substances (3-4) in marine organisms, we found that certain Sea pen members (§) contain potentially important antineoplastic constituents.

We now have isolated three new 21-membered-ring lactones **1**, **2** and **3**, named **Lituarines A, B,** and **C**, from extracts of the New Caledonian Sea pen : *Lituaria australasiae* . As Lituarines could not be obtained in crystalline state suitable for X-ray crystal structure determination, unequivocal assignment of structures **1**, **2** and **3** was performed by 1D and 2D ^{1}H and ^{13}C NMR studies. NMR multipulse sequences used were COSY, TOCSY, NOESY, ROESY and DEPT.

Lituarines are the first macrocyclic lactones with two fused tetrahydropyran rings reported from marine sources.



Lituarines

	R_1	R_2
1	H	H
2	$OCOCH_3$	OH
3	OH	OH

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(§) Acknowledgment : we wish to thank Marie-José D'HONDT (Museum National d'Histoire Naturelle) for the taxonomic study of *L. australasiae*, Georges BARGIBANT and Jean-Louis MENOU for collection assistance and Professor Jean-François VERBIST (faculté de Pharmacie de Nantes) for Bioassay.

Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

Marine chemical ecology. Chemicals speak softly in all languages

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In this lecture, we would like to illustrate the way secondary metabolites play multifunctional roles in the ecology of marine organisms. Special emphasis will be placed on the roles played by molecules contained in, and released from the tissues of the Alcyonacean soft corals genus *Sinularia.*

Third Pacific-Asia Symposium on biologically active natural products, Nouméa, New Caledonia, August 26-30th, 1991

Phylogenetic specificity of *Conus* neuropeptides

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The venom of the predatory cone snails (*Conus*) contain a diverse set of biologically active peptides. In addition to the major toxins used to incapacitate or paralyze prey, many other neuroactive peptides have been found in all species examined. Although certain class of toxins are found in related species, each species has a unique set of peptides.

Seven α–conotoxins (inhibitors of acetylcholine receptors), 11 ω–conotoxins (blockers of voltage sensitive calcium channels), and 3 conantokins (inhibitors of N-methyl-D-aspartate receptors) have so far been isolated from five *Conus* species. The conotoxins are small basic peptides ranging from 13 to 29 amino acids long with 2 to 3 disulfide bonds. On the other hand, conantoxins are very acidic peptides containing 4 γ-carboxyglutamates and no cysteine residues. Presumably these peptides are targeted to receptors and ion channel of their prey and natural enemies but they can also recognize some targets in other vertebrates.

The α-conotoxins from *Conus geographus* and *Conus magnus* cause paralysis when injected intraperitoneally in fish, frog, chicks and mice but two of the α-conotoxins from *Conus striatus* affect only the fish neuromuscular junction at a comparable dose. The ω–conotoxins are also very effective in paralysing fish but they affect only certain subtypes of calcium channels in the mammalian central nervous system. Thus, instead of causing death when injected intracranially, the ω–conotoxins produce a very characteristic shaking symptom in mice and rats. Conantokins induce behavioral symptoms dependent on the developmental stage of mice: they cause sleep in two-week old mice and hyperactivity in mice greater than three week old. In fish they produce depressed activity.

Structure-activity comparison of naturally occuring and synthetic analogs of α-conotoxins, ω–conotoxins and conantokins indicate the importance of certain residues in the recognition of target molecules in the different species. The homologous set of peptides from *Conus* venoms are now used as biochemical probes for receptors and ion channels.

Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

Indole alkaloids from two New Caledonian tunicates, *Eudistoma album* and *Pseudodistoma arborescens*

Cécile Debitus, J.-B. Carré, M. Païs*, M. Chbani*, G. Bargibant, J.-L. Menou , P. Tirard et D. Duhet

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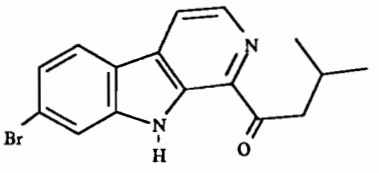
During our work on marine organisms from New Caledonia, we studied some of the major species of tunicates, together with the zoological survey of the marine Fauna from the New Caledonian lagoon (1) (2).

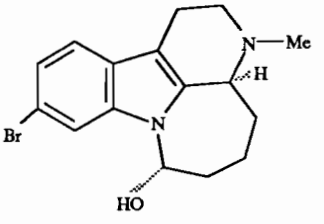
34 species were extracted and screened on several bioassays; 8 of them showed reliable interesting bioactivity and have been further chemically studied. Among these selected organisms, two species of *Eudistoma* (Polycitoridae) and *Pseudodistoma arborescens* (Polyclinidae) yielded some new indole alkaloids.

Woodinine, a new tetrahydro-ß carboline has isolated from *Eudistoma fragum* (3) and a recent work led to the isolation of the new alkaloid Eudistomin U together with the antiviral Eudistomin E from an other *Eusdistoma sp., E. album..*

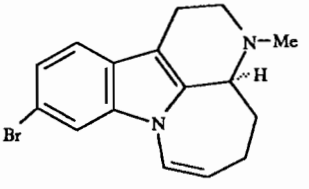
Pseudodistoma arborescens belongs to a different family of Ascidians, but has been studied also for its antimicrobial and cytotoxic properties: three new ß-carbolines named Arborescidines A, B and C were identified, thought they are not the bioactive metabolites, which are still being studied.



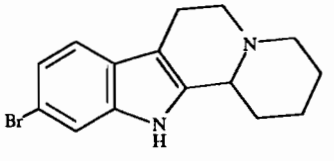
Eudistomine U



Arborescidine C



Arborescidine A



Arborescidine B

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Third Pacific-Asia Symposium on biologically active natural products, Noumea, New Caledonia, August 26-30th, 1991

Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

How to corroborate a spinal mechanism of action for a drug. Example of the hypotension induced by a dopamine receptor agonist ?

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An increasing number of neurotransmitters are shown to be involved in spinal transmission. Their presence is corroborated with techniques such as chemical titration or staining (for example by immunocytochemistry) of the considered mediator or of its synthesis enzyme; it is also possible to show a basal or stimulation-induced release of this transmitter by microdialysis or superfusion techniques.

Their sites of action can be evidenced by radioligand binding techniques, either on tissue homogenates or by quantitative autoradiography, this latter methodology allowing to specify, besides their density and affinity, the localization of the binding sites.

The biological functions mediated, at least in part, by these spinal transmitters, can be studied by the changes induced by intrathecal (i.t.) administrations, at different levels, of agonists or antagonists, either in normal or spinalized animals. The results of these i.t. administrations, either rostrally or caudally with respect to the transection level, will depend on the way, that is ascending or descending, of the spinal transmission. In addition, the destruction by a specific neurotoxin of the spinal neurotransmitter system involved in the considered function, can give further elements supporting this involvement.

As an example of this methodology, the experimental results supporting a partial spinal mediation for the hypotensive effects of bromocriptine are presented herein.

Strong evidence has been provided, by biochemical and anatomical studies, for the identification of a descending diencephalo-spinal dopaminergic system (DDSS) and, among the effects induced by an injection of apomorphine, a dopamine receptor agonist, in the spinal subarachnoidal space, at doses to low to be active by i.v. administration, it can be observed hypotensive and bradycardic effects.

These effects appear immediately after i.t. administration of apomorphine; furthermore, their magnitudes and times of appearance are different according to the level of administration along the rostro-caudal axis and the distribution studies of tritiated apomorphine i.t. injected support the hypothesis of a spinal localized area for the origin of these cardiovascular effects.

Bromocriptine, a D2 receptor agonist, is used clinically in the treatments of hyperprolactinemia and Parkinson's disease; in addition it was found to decrease blood pressure and the mechanism of this hypotensive effect is still not fully understood . So we studied the possible participation of the DDSS in the hypotensive effect of i.v. administered bromocriptine by the evaluation of the antagonism induced by domperidone (a dopamine receptor antagonist unable to cross the blood-brain barrier) injected either by i.v (peripheral blockade) or i.t.(spinal blockade) route. It was shown, in conscious or pentobarbital-anesthetized normotensive rats that about 35 % of the whole hypotensive response was elicited by spinal mediation. The tachycardia induced by bromocriptine seemed not to be of a dopaminergic nature.

As a complete spinal transection induced caudally, but not rostrally, an increase in the i.t. injected apomorphine-induced hypotension and bradycardia, we performed the same experiments as described above in spinal rats; it was observed that magnitude of the hypotension induced by i.v. bromocriptine was increased. The peripheral part (antagonism by i.v. domperidone) of this hypotension was unchanged and it was the spinal part (antagonism by i.t. domperidone) which was increased. This result in spinal rat may help to explain the increased orthostatic hypotension induced by bromocriptine in parkinsonian patients with spinal lesions.

Third Pacific-Asia Symposium on biologically active natural products, Noumea, New Caledonia, August 26-30th, 1991

Application of radioreceptor binding analysis to the detection of marine natural products with therapeutic potential

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Radioreceptor binding techniques have the potential to readily detect novel activities within natural products. The method involves coincubation of extracts with a membrane preparation containing the receptor of interest and a suitable radiolabelled ligand. A subsequent decrease in the amount of bound radiolabel may indicate the presence of a competing ligand within the extract. Confounding results will be obtained when the extract interacts indirectly with the receptor preparation or radiolabelled ligand. Despite some potential for non-specific interactions, this technique has the opportunity to identify novel natural products with a specific mechanism. This approach contrasts with those employing animate systems as whole animals or cultured cells in which a broad spectrum of mechanisms including general toxicity may be detected. Particularly with marine organisms, the high incidence of toxic metabolites may ask the detection of other interesting biological activities.

In our laboratory, we employ radioreceptor binding assays for the epidermal growth factor ([^{125}I] EGF), growth hormone ([^{125}I] GF) and glutamate excitatory amino acid neurotransmitter ([^{3}H] glutamate, [^{3}H] kainate) receptors. Initial screening of marine extracts of samples collected throughout Australasia resulted in a positive result rate of ~1%. Non-specific effects were controlled against by comparison between receptor assays. Specific leads are pursued using assay guided purification.

The utility of radioreceptor binding methods in the detection and purification of marine metabolites will be presented using data from the ligands listed above.

Cytotoxicity studies for the detection and quantification of maitotoxin (MTX).

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Maitotoxin (MTX) is the principal toxin produced by the benthic dinoflagellate *Gambierdiscus toxicus* in culture. The detection and quantification of this molecule of unknown structure is conducted with a biological test, the mouse bioassay being a reference test commonly used. The amount of toxic extract necessary to kill a 20 g mouse in 24 h is defined as a Mouse Unit (MU). Cytotoxicity measurements can complement and partially replace such a test. In order to detect and quantify MTX throughout the different steps of purification, the eventual toxicity of extracts was assayed upon mouse and different types of mammal cells in culture. The 50% inhibitory concentration (IC50) of MTX was evaluated on fibroblastic (L929, 3T3, FR 3T3, BHK-21 C 13) and neuroblastic (N18) cells using cell counts, and the colorimetric test MTT and the neutral red assay at 3, 24 and 48 h after exposure of the cells to the extracts. The sensitivity of the cells varied according to the cell type from 0.001 to 0.1 MU/ml. Detection was possible through observation under an inverted microscope 1 hour after exposure and quantification after 3 hours. The neutral red assay is currently conducted in our laboratory with a multi-well plate absorbance detector that allows a rapid screening of a large number of fractions obtained throughout the different steps of MTX purification.

Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

Mise au point sur les substances "nématicides" produites par des microorganismes et des végétaux supérieurs

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Les nématodes phytoparasites constituent un problème majeur pour l'agriculture au plan mondial car ils s'attaquent aussi bien aux grandes cultures qu'aux cultures maraîchères et florales, les dommages étant estimés annuellement à 77 milliards de dollars. Outre les assolements, la mise en jachère ou l'inondation des sols infestés et l'emploi de variétés résistantes ou d'agents de lutte biologique (champignons nématophages) qui restent encore des cas exceptionnels, les seuls moyens de lutte actuellement disponibles consistent à désinfecter les terrains contaminés à l'aide de produits nématicides gazeux dérivés des gaz de guerre qui sont extrèmement dangeureux pour l'homme et son environnement. Mis à l'index par les écologistes, ces nématicides chimiques sont progressivement interdits d'emploi, ce qui incite partenaires privés et publics à s'orienter vers la recherche de nouvelles molécules "nématicides" (comprenant les substances nématifuges, nématostatiques et nématicides au sens strict) non polluante d'origine naturelle.

A partir de recherches sur les antibiotiques, un certain nombre de principes "nématicides" ont été mis en évidence chez des **bactéries** (genres *Bacillus* et *Pseudomonas*) et chez des champignons (genres *Actinomadura* et *Streptomyces*). De récents travaux menés au laboratoire de Nématologie de l'INRA d'Antibes sur l'étude des propriétés nématicides de filtrats de culture de champignons ont également permis d'isoler et caractériser les principes toxiques en cause; la recherche de l'explication de la spécificité et du mode d'action de molécules dérivées (brevet INRA) est actuellement à l'étude de même que la mise en évidence de l'activité nématicide de 2 dérivés de la famille des Avermectines (lactones macrocycliques) produits par *Streptomyces avermitilis* et déjà utilisés comme anthelminthiques, insecticides et acaricides.

Chez les **algues** et les **spongiaires**, on connaît encore peu d'espèces à vertu nématicide (*Spatheoglossum schroedi, Phormidium tenue, Astorionella japonica*).

La production de substances "nématicides" par des **végétaux supérieurs** est par contre connue depuis très longtemps. Ces substances peuvent être exsudées au niveau des racines et agir soit en inhibant l'éclosion des larves dans les racines (exsudat ovicide de la moutarde *Sinapis alba*), soit en inhibant la pénétration des larves dans les racines (exsudat répulsif de *Tagetes minuta* ou larvicide de *Catharanthus roseus*). Elles peuvent aussi être synthétisées lors de l'infestation (phytoalexines telles que la glyceolline du soja cv centennial) ou être déjà présentes dans les tissus au niveau des tiges, feuilles, fleurs, graines ou racines et agir en empoisonnant la larve (*Vernonia polyanthes*), soit en bloquant son développement (*Ricinus communis*).

Certaines de ces plantes sont introduites dans des rotations culturales en cultures intercalaires et utilisées comme engrais verts nématicides; ce sont surtout des Fabaceae (*Crotalaria sp., Stylosanthes sp.*) mais aussi des Asteraceae (*Tagetes sp., Cosmos sp.*) et des Poaceae (*Eragrostis sp.*).

De nombreuses pratiques indigènes en Afrique, en Inde, aux Philippines et en Amérique du Sud les utilisent également (surtout plantes herbacées ou ligneuses) sous forme de préparations à base de broyats (racines d'*Euphorbia hirta*, tiges de *Vernonia polyanthes*, *Ricinus communis* , feuilles de *Calotropis gigantea* , fleurs de *Tagetes minuta*, graines d'*Azadirachta indica*) ou de tourteaux (*Azadirachta indica, Gossypium arboreum, Pongamia glabra*...) qui sont incorporés aux sols cultivés et servent d'amendements organiques nématicides. L'analyse des substances produites lors de la décomposition de ces matières organiques dans les sols a permis d'identifier, outre les éléments N, P, K qui stimulent l'activité des parasites naturels ou prédateurs des nématodes, différents acides gras volatils (acides formique, acétique, propionique et butyrique principalement) qui seraient en partie responsable de l'activité "nématicide" des amendements.

Certains travaux sommaires au Brésil mais surtout en Inde ont consisté à tester in vitro les extraits aqueux, alcooliques et lipidiques des différents tissus d'espèces appartenant à des taxons très variés (Amaranthaceae, Asteraceae, Brassicaceae, Solanaceae...) sur les œufs et larves de divers nématodes, principalement *Meloidogyne sp.*, nématode à galles des racines, le plus répandu et causant les plus gros dégats dans le monde.

Des investigations plus poussées (en Californie notamment) ont déjà permis l'isolement et la caractérisation d'un certain nombre de principes actifs. Les structures chimiques de ces substances "nématicides" s'étendent très largement des polyacétylènes (d'*Angelica pubescens*), acides (d'*Asparagus sp.*), esters carboxyliques (d'*Arachis hypogeae*), acides gras insaponifiables (d'*Iris japonica, Cyperus esculentus*), acides aminés (de *Sesamum orientale, Abelmoschus esculentus*), et protéines (de *Canavalia ensiformis, Mucuna deeringiana*), aux composés aromatiques (d'*Hedera helix, Eragrostis curvula*), hétérocycles à oxygène (de *Nyctanthes arbortristis*), ou à soufre (de *Tagetes sp.*), alcaloïdes (de *Bocconia citratus, Vernonia colorata, Daphne odora, Gossypium arboreum, Artemisia maritima*).

Il a été mis en évidence que la plupart de ces substances naturelles "nématicides" sont décomposables et agissent spécifiquement sur certaines espèces de nématodes phytoparasites; elles peuvent être utilisées comme base pour la synthèse de nouveaux nématicides, moins polluants et respectant les espèces utiles du sol.

Third Pacific-Asia Symposium on biologically active natural products, Noumea, New Caledonia, August 26-30th, 1991

New diterpenes from blackcurrant buds (*Ribes nigrum* L.)

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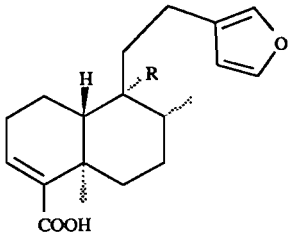
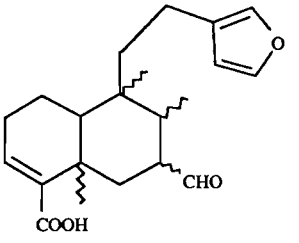
The presence of large amounts of diterpene acids in French blackcurrant bud extract was first reported by Fellous *et. al.* (1). They identified (+)-hardwickiic acid as the major acid identical with (+)-hardwickiic acid isolated from *Copaifera officinalis* (2). More recently, Derbesy *et.al.* (3) confirmed the presence of (+)-hardwickiic acid (34%) and isolated another acid (II)(11%). The absolute configuration of neither of these was specified.

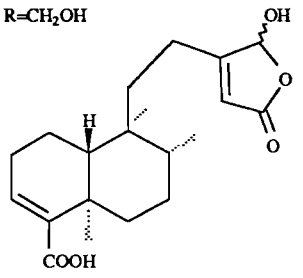
In contrast, Tasmanian blackcurrant bud extracts contain the **laevorotatory** isomer (-)-hardwickiic acid (I, R=CH_3, 32%) which also occurs in *Hardwickia pinnata* (4). In addition, a series of five previously unreported compounds have been isolated. Three of these are derivatives of (-)-hardwickiic acid oxygenated at the methyl group attached to C9. The aldehyde (I, R=CHO, 16%) is a major component with the diacid and hydroxyacid occuring in small amounts. Two isomeric γ-hydroxybutenolides (III) and (IV) were also present in minor amounts. These two compounds presumably arise from oxidation (5) of the furan ring of (-)-hardwickiic acid. No trace of compound (II) was detected.



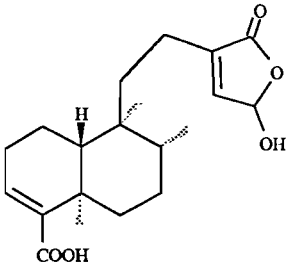
(I) R=CH_3 (-)-Hardwickiic acid
R=CHO
R=COOH
R=CH_2OH



(II)



(III)



(IV)

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Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

Biochemical and electrophysiological characterization of the toxic component from the venom of the greater weever fish *Trachinus draco*

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The venom of the greater weever fish, *Trachinus draco* was analysed to determine its toxicity, stability and biological properties. Crude venom prepared from the venom apparatus of one fish had a MLD of about 2 µg venom protein per g mouse. The venom caused a significant tetraphenylphosphonium (TPP) release from preloaded rat brain particles. The venom also possessed hemolytic activity with EC_{50} of 75 ng/ml for rabbit erythrocytes. The hemolytic component of the venom was purified to near homogeneity by ammonium sulphate precipitation followed by HPLC. The purified hemolysin had a molecular mass of 105 kDa and an EC_{50} of 3 ng/ml with rabbit erythrocytes.

Since the purified hemolysin looses quickly its biological activity under physiological conditions, we had to use the crude venom for electrophysiological experiments. At motor endplates of *M. triangularis sterni* of mice the venom (5-50 µg/ml) caused presynaptically a massive quantal release of acetylcholine and postsynaptically a strong decrease of the membrane potential followed by damages of nerve terminals and muscle fibres. In isolated outside-out membrane patches from bovine adrenal chromaffin cells single channel currents evoked by the venom have been recorded. The single channel conductance of the largest pores is 2500 pS. These pores are nonselective for mono- and divalent cations. The existence of 3 to 5 subconductance states each of about 500 pS indicates the heterogeneity in pore formation and may be due to pores composed of 3 to 5 monomers.

From our data we conclude that the venom of the greater weever fish possesses hemolytic and cytotoxic activity which is related to a single component of the venom and which is most probably responsible for the clinical symptoms and the lethal activity.

Third Pacific-Asia Symposium on biologically active natural products, Noumea, New Caledonia, August 26-30th, 1991

L'austrocalédonine, nouvel alcaloïde extrait d'un conifère endémique de Nouvelle-Calédonie, *Austrotaxus spicata*

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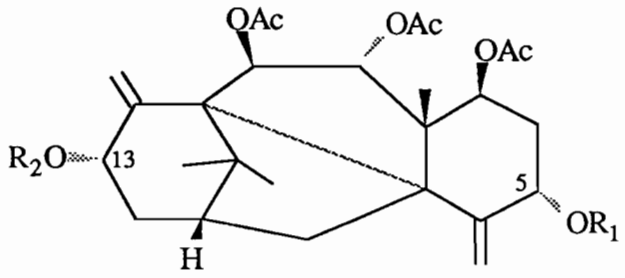
Vingt-neuf composés de type taxane ont déjà été isolés par nos soins des feuilles et des écorces d'*Austrotaxus spicata* Compton (Taxacées): vingt-deux sont des alcaloïdes diterpéniques, sept des diterpènes "neutres".

Deux nouveaux composés, minoritaires, viennent d'être isolés et identifiés : l'un d'eux est un diterpène de type taxane 1, l'autre, un alcaloïde 2, appelée austrocalédonine, possédant le même squelette, mais substitué en 5α par une chaîne 2'-hydroxy-3'diméthylamino-3'-phényl propionate et en 13α par un acétate.



1 $R_1=R_2=H$

2 R_1=CO-CHOH-CH(NMe_2)-Ph
$R_2=COCH_3$

Marine natural products from the Seychelles

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Approximately 100 specimens of sessile marine organisms were collected by hand using SCUBA along the coastlines of granite islands around Mahé in the Seychelles, which are a diverse group of islands located in the western equatorial Indian Ocean. The crude methanolic extract of each organism was evaluated in antimicrobial and cytotoxicity assays and by examination of the ^{1}H NMR spectrum. The bioactivity data was unexceptional but the ^{1}H NMR spectra revealed several unusual compounds.

A dark-green tunicate, *Eudistoma* sp., was collected at Praslin Island. The major metabolite of the tunicate was ascididemnin, which had previously been isolated from a tunicate of the genus *Didemnum* by Kobayashi *et al.* Among the minor constituents were two octacyclic alkaloids, eudistones A and B. The structural elucidation of eudistones A and B was based on a detailed analysis of ^{1}H and ^{13}C NMR data.

A sponge of the genus *Smenospongia* was collected at Therese Island. The sponge was chosen for study because the crude extract gave an unusual ^{1}H NMR spectrum. A ^{1}H NMR guided fractionation led to the isolation of four chromene derivatives. The smenochromenes A-D are macrocyclic chromenes that can be derived by cyclization of farnesyl hydroquinone. Although the structure of smenochromene A could have been determined by interpretation of spectral data, the structure had to be confirmed by X-ray analysis because the unusual geometry of the compound led to unexpected spectral data. The spectral data and conformations of the smenochromenes will be discussed in detail.

New antitumor-antibiotics from marine microorganisms

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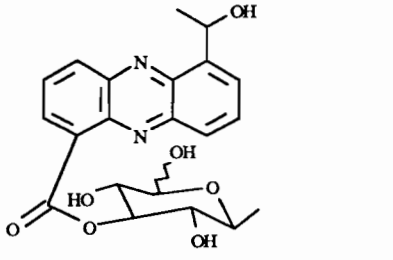
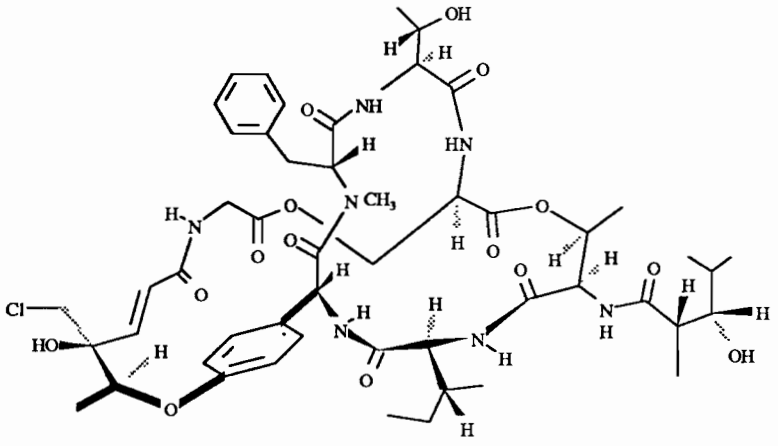
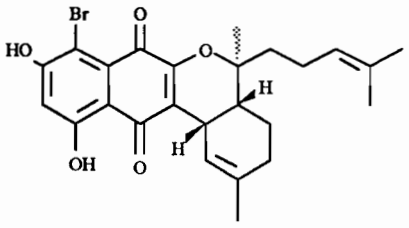
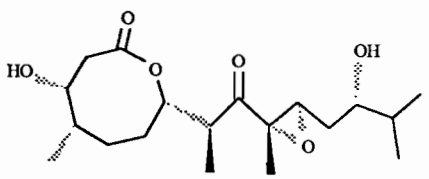
Historically, soil microorganisms are the single major source for chemotherapeutic agents. Over 110 products from these fermentation sources are in clinical use today to treat a wide variety of diseases. Although soil-derived microorganisms are still intensely studied, new microbial resources will be essential if fermentation is to continue to satisfy the need for new drugs. It is in this regard that we are now exploring microbiological resources from marine habitats. In preliminary studies of marine bacteria, we have focussed on developing new methods of isolation and culture of a wide diversity of marine-derived microorganisms. One focus of our work has been the discovery of new antibiotics and antitumor agents of novel architectures. Compounds such as those below represent recent discoveries of new classes of bioactive agents. Details of the microorganism exploration process, the culture and screening of marine microorganisms and of the structure determination of these compouds will be discussed.









The use of sea urchin egg as a model to investigate the cellular targets of marine natural products

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Sea urchin gametes are, among many invertebrates gametes, one of the most sensitive cells to environment stress. Since 1980, many authors have widely described the properties of cultured sea urchin eggs and embryos resulting in several advantages over conventional mammalian cultured cells. Cultured in simple sea water, sea urchins provide millions of eggs which can be successfully fertilized. Cell divisions remain synchronous at least until the third cleavage, which occurs within a few hours. Thus, it is easy to rapidly know, under a light microscope, if a drug affects the fertilization ratio or the cell cycle, which can be delayed or blocked.

Among drugcellular targets, the role played by the plasma membrane and the membranes of intracellular organelles has been widely studied 1) maintenance of ionic equilibrium; 2) ionic signals following activation by sperm and 3) stimulation of metabolic processes resulting in the first cleavage and embryogenesis. Sperm triggers resumption of the egg activity, the first round of DNA synthesis being achieved 30 min. later. Like in other animal cells, activation starts by rapid hydrolysis of phosphatidylinositol-biphosphate generating the phosphoinositide messengers inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 triggers an explosive wave of cytosolic calcium and DAG provokes a further sustained increase in intracellular pH. The calcium increase during fertilization is the necessary and sufficient signal that induces egg development. In addition, other calcium transient changes occur throughout the first cell cycle particularly at anaphase and before cleavage.

As previously proposed by us, drug inhibition of sea urchin egg fertilization and first cleavage can be explained in many cases by changes of ion transport through the plasma and subcellular membranes. Many types of drugs can be characterized depending on their cellular effects and their targets. Inhibition of the Na^+/H^+ exchange alters the sustained alkalinization of egg after fertilization and the mechanism by which the egg maintains an intracellular pH below that of sea water. After fertilization, inhibition of the Na^+/K^+ exchange increases cell sodium content, inhibits amino acids uptake and leads to vegetalized embryos. Calcium permeability and calcium sequestration by intracellular organelles such as endoplasmic reticulum or mitochondria can also be modified by drugs. A rise in calcium permeability of the plasma membrane can result in a calcium increase which in turn may uncouple mitochondrial metabolism, both events leading to cell death. Drug-induced calcium leakage from intracellular stores, mainly endoplasmic reticulum, elevates cytosolic calcium concentration and can prevent egg from undergoing later calcium transient changes. In addition, sea urchin eggs offer the possibility to easily examine drug effects on the cytoskeleton by observing evolution of the mitotic spindle and the cleavage furrow. With sea urchin eggs, it is therefore possible to consider the relations between intracellular calcium, pH and the behaviour of the cytoskeleton.

Chemical and bioactive principles from selected Malaysian plants

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Some aspects of current studies on Malaysian rainforest plants as well as those used in Malaysian traditional medicine will be reviewed including the following : phytochemical screenings, isolation and characterisation of new natural products, antihypertensive natural products, DNA-cleaving compounds and tumor-promoting chemicals.

Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

Métabolisme et pharmacocinétique des alcaloïdes de l'ergot de seigle : apport de l'imunologie analytique

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Les alcaloïdes peptidiques extraits de l'ergot de seigle (*Claviceps purpurea*), connus et utilisés depuis plus d'un siècle, et surtout leurs dérivés d'hémisynthèse sont toujours très présents dans l'arsenal thérapeutique moderne. Ils occupent des places importantes au sein de classes telles que les utérotoniques (méthylergométrine), les anti-migraineux (ergotamine, dihydroergotamine) et les anti-parkinsoniens (bromocriptine). Ils sont prescrits pour lutter contre les hyperprolactinémies (bromocriptine, métergoline) et contre les troubles liés à la sénescence cérébrale (nicergoline, dihydroergotoxine).

Les taux plasmatiques atteints en cours de traitement sont en règle générale compris entre une dizaine et une centaine de pg/ml. Ces concentrations sont le reflet d'un volume de distribution élevé, d'une forte métabolisation et d'une biodisponibilité orale souvent réduite. En conséquence, ces molécules ont toujours posé un problème à l'investigateur qui, pour les identifier et les doser dans les fluides biologiques a besoin de disposer de méthodes toujours plus spécifiques et sensibles.

La technique immuno-analytique a dans ce domaine permis d'obtenir des outils performants. Diverses stratégies analytiques ont été développées aussi bien en radio-immuno-analyse (RIA) qu'en enzymo-immuno-analyse (EIA) afin d'obtenir des méthodes de détection permettant le dosage des molécules non transformées et/ou de leurs métabolites actifs ou inactifs. Ces approches nécessitent l'alliance de techniques de synthèse organique, d'immunochimie et de radio-chimie.

Les dosages immuno-analytiques permettent le calcul des paramètres d'absorption, de distribution et d'élimination de ces composés chez l'homme et contribuent donc à la mise au point et à la comparaison de formes pharmaceutiques nouvelles.

Ces méthodes ont été mises en oeuvre pour suivre le métabolisme de la dihydroergotamine par diverses isoenzymes du cytochrome P450 hépatique. Le mécanisme de l'interaction médicamenteuse avec les antibiotiques macrolides a ainsi pu être précisé.

La recherche de relations entre les taux plasmatiques des alcaloïdes (ou de leurs métabolites) et les effets pharmacodynamiques observés chez l'homme peuvent être modélisés et servir à l'optimisation des conditions d'administration.

Third Pacific-Asia Symposium on biologically active natural products, Noumea, New Caledonia, August 26-30th, 1991

Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

Tubuline-microtubules system and the research of new antimitotic drugs.

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One of the aim of our laboratory is to find new highly active spindle poisons of natural or synthetic origin. To detect this potential antitumor activity, we use a simple acellular assay called the tubulin test.

In vivo, microtubules fulfill different functions in cells : besides to be the major constituant of the spindle apparatus, they are essential for the generation of movement by cilia and flagella and they play a key role during axoplasmic transport, cytoskeleton framework and in cell secretion. Microtubules are formed by assembly of protein subunit called a and b tubulin.

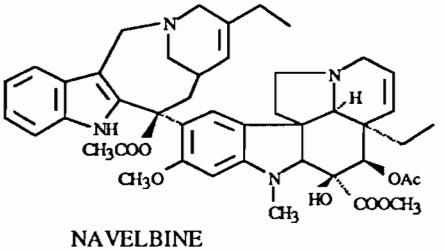
In vitro, tubulin can be easily purified from fresh mammalian brain and its activity monitored by a UV spectrophotometer equipped with a thermstated cell. The rate of assembly (37°C) or disassembly (0°C) is checked for each drug or mixture at different concentration.

Thus, the tubulin assay is a very efficient tool to detect new potential antitumor substances and from the time we developped this *in vitro* assay in our laboratory, we discovered the antimitotic activity of known compounds and by structure activity relationships studies, new natural or synthetic compounds were selected for pharmacological assay as cytotoxicity.

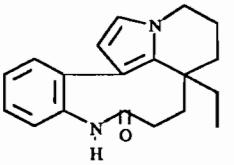
For example in the vinblastine-type compounds, this tubulin assay allowed the selection of Navelbine, a new synthetic antitumor vinca-alkaloids which is now in clinical use (1).

In the taxol family, the use of this test led first to the isolation of a large number of new natural active analogs of taxol and next allowed the selection of taxotere for the beginning of the pharmacological and clinical assays (2).

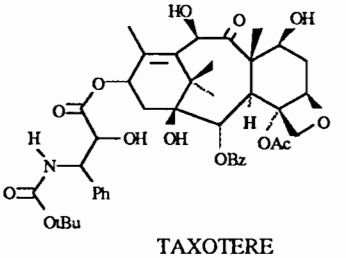
Recently, from an ethanolic extract of the trunk bark we found a known substance, rhazinilam, with a new activity on the tubulin-microtubules system : it induces the spiralization of tubulin dimer as vinblastine and it inhibits the disassembly of microtubules as taxol. Rhazinilam is the prototype of a new class of antitubulin compounds (3).



NAVELBINE



RHAZINILAM



TAXOTERE

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Third Pacific-Asia Symposium on biologically active natural products, Noumea, New Caledonia, August 26-30th, 1991

Lissoclinotoxins, antibiotic 1,2,3-trithiane derivatives : structure and synthesis.

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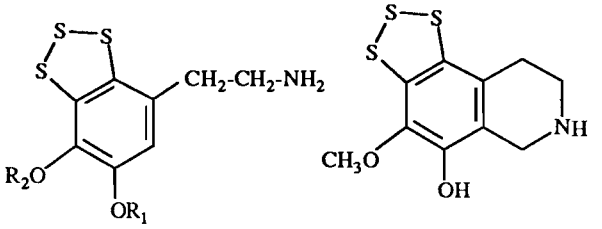
Lissoclinotoxins A and B have been isolated from the tunicate *Lissoclinum perforatum* collected in Dinard (France).

Structure 1a was proposed from spectral data for lissoclinotoxin A (1). However, long range C-H N.M.R. correlations led us to revise the proposed structure of lissoclinotoxin A and to assign structure 1b.

By comparison, structure 2 was proposed for lissoclinotoxin B.

A total synthesis starting from vanillin (for 1a) and isovanillin (for 1b) will be described.



1a : R_1 = OMe , R_2 = OH

1b : R_1 = OH, R_2 = OMe

Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

Natural products from Malaysian plants

Hamid A. Hadi

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The flora of Malaysia is generally considered to be one of the richest in the world. Up to the present very few investigations of the chemical composition of Malaysian plant species have been made. Aware of the considerable interest of this endangered flora, an independently financed collaborative programme concerning phytochemical research of flora of Malaysia (Peninsula, Sabah and Sarawak) was set up in 1981 between ICSN, CNRS, Gif-sur-Yvette, France and Department of Chemistry, University of Malaya, Kuala Lumpur.

This programme involved field trips and plant collection according to botanical classifications, field testing followed by collection of raw material in large quantities when a preliminary test was positive. Some chemical studies on the plants collected have been performed either by the Malaysian or French partner.

Many alkaloids and other natural products including new compounds have been isolated and elucidated from various plant species. Some pharmacological studies have also been done on Malaysian plants. In order to search for new anticancer substances, we have performed cytotoxicity test and antitubulin activity on ethanol extracts of plants collected. The result of these activities will be discussed.

Third Pacific-Asia Symposium on biologically active natural products, Noumea, New Caledonia, August 26-30th, 1991

Chemical and biochemical studies on the biologically active natural products in Korea

Byung Hoon Han

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More than 3500 plant species are found in Korea, located in northern temperate zone. these plants have been used traditionary in primary health care as the folklore medicine. Many korean scientists are investigating on the pharmacological efficacy and on the phytochemical components of the plants.

Bio-activities are monitored using various in vitro and in vivo assay techniques. To explore some new lead compounds for new drug development from plant sources, these approaches are highly recommended in Korea since we have abundant folkloric experiences on the efficacy and the toxicological aspects of the plants. A few successful achievements in my laboratory and others in Korea will be described in this talk. My present talk will include the following topics : researches on 1) Sedative cyclopeptide alkaloids from Rhamnaceae plants, 2) antioxydant and antifatigue phenolic components of Korean *Panax ginseng* , 3) Antiinflammatory diterpenoids of some Araliaceae plants.

Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

Isolation and synthesis of biologically active naphtoquinone derivatives from a plant source.

Banasri Hazra, S. Pal and A. Banerjee

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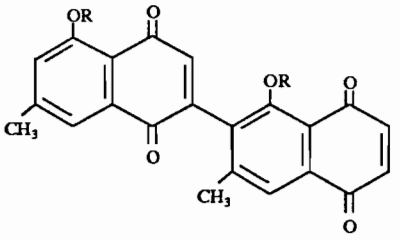
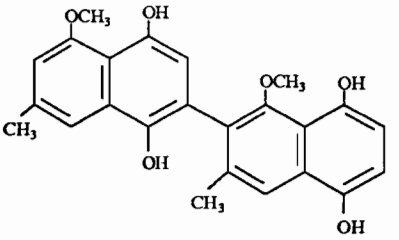
In continuation of our work on the antitumour [1] and antileishmanial [2] activities of diospyrin (**1**) isolated from *Diospyros montana* Roxb., it was our curiosity to attempt suitable structural modifications of this *bis*-naphtoquinone derivative, so that its cytotoxicity towards the host *in vivo* could be reduced with a concomitant enhancement in its chemotherapeutic activity. Diospyrin dimethyl ether (DDE; **2**) and tetrahydroxydiospyrin dimethyl ether (TDDE; **3**) were synthesized, identified by analytical and spectroscopic procedures, followed by investigations on their biological activities.



1 R=H 2 R=CH_3



3

Antitumour activity against Ehrlich Ascites Carcinoma in Swiss albino mice was studied by evaluation of various parameters, such as survival time and tumour growth, followed by haematological, biochemical and histopathological investigations. The dose-regimen for each drug injected intraperitoneally to the "treated" group was standardized by repeated trial and error. It was observed that: (i) synthetic derivatives could increase the life span of "treated" mice by more than 100% with respect to the "control" group, as compared to less than 50% with diospyrin itself; (ii) the effective dose for TDDE (3) was about half of that for DDE (2); (iii) the cytotoxicity of the synthetic derivatives towards the host system was considerably less than of diospyrin itself, as indicated by the haematological, biochemical and histopathological data.

The antiprotozoal activities of the naphtoquinones were studied against *Leishmania donovani* promastigotes in liquid culture media. It was found that the synthetic derivatives were more effective antileishmanial agents than the natural product itself.

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Funded by the University Grants Commission, India and the International Foundation for Science (Grant No. F/1836-1), Sweden

Third Pacific-Asia Symposium on biologically active natural products, Noumea, New Caledonia, August 26-30th, 1991

Macaranga vedeliana, une Euphorbiaceae utilisée en médecine empirique en Nouvelle-Calédonie

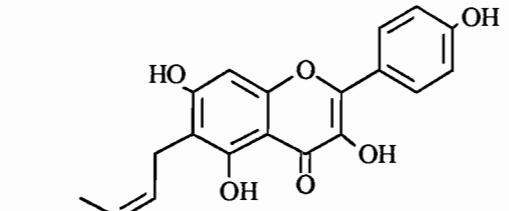
Edouard Hnawia, O.Thoison*, F.Guéritte-Voeguelein*, J.P.Riffaud**, J.M.Veillon***, D.Bourret****, T.Sévenet*

*Université Française du Pacifique, Nouméa, Nouvelle-Calédonie; *ICSN-CNRS, Gif-sur-Yvette, France; **Laboratoires DEBAT, Garches, France; *** Centre ORSTOM de Nouméa, Nouvelle-Calédonie; **** ORSTOM, La Réunion*

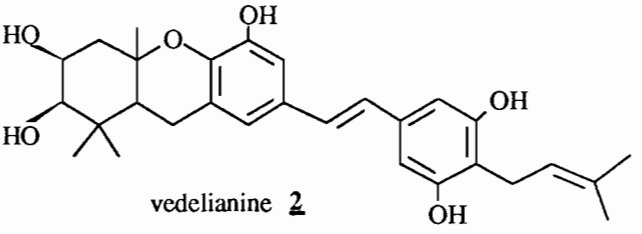
Dans le cadre d'une collaboration interdisciplinaire, l'étude chimique et pharmacologique de plantes utilisées en médecine traditionnelle en Nouvelle-Calédonie a été entreprise. Parmi les plantes utilisées contre la douleur, les rhumatismes, les coups et les blessures, plusieurs Euphorbiaceae ont été récoltées et testées dans un criblage large.

Parmi ces plantes, *Macaranga vedeliana* (Baill.) Muell.-Arg.), utilisé en Nouvelle-Calédonie pour soigner les rhumatismes, n'a pas révélé, dans les tests pharmacologiques pratiqués sur le rat, d'action analgésique et antiinflammatoire, mais une activité hypotensive nette, avec diminution de la fréquence cardiaque.

Le fractionnement chimique a permis d'isoler, à côté de flavones bien connues, la macarangine **1**, le diisoprénylkaempférol et la vedelianine **2**.



Macarangin **1**



vedelianine **2**

Référence:

E.Hnawia, O.Thoison, F.Guéritte-Voegelein, D.Bourret and T.Sévenet , Phytochemistry, 29 (7), 2367-2368 (1990)

Ardisiacrispin B, a prostaglandin-like effect saponin, isolated from *Ardisia crispa*

Chaweewan Jansakul

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Prostaglandin E_2 is the most potent agent for pregnancy termination (Conrad & Ueland, 1979; Rayburn, 1989). However, the limitations of the compound are its instability and high price. In 1987, Jansakul *et al.* reported that Ardisiacrispin B, one of the two major saponin isolated from *Ardisia crispa* showed a pronounced contraction on rat uterine smooth muscle in isolated preparation.

The present study aimed to characterize the pharmacologic type of the Ardisiacrispin B. Studies were performed *in vitro* preparation, using uterine smooth muscle, small intestine and thoracic aortae (vascular smooth muscle) obtained from female rats in estrus. Dose response-curve (DR-curve) to Ardisiacrispin B, Prostaglandin E_2 derivative (Nalador), Oxytocin and Acetylcholine were obtained. The possible involvement of prostaglandin synthesis in the utero-contracting activity of Ardisiacripin B were also explored by investigation of the DR-curve to Ardisiacrispin B in the presence of 10^{-6} M indomethacin, a cyclo-oxygenase inhibitor.

Ardisiacrispin B caused contraction in dose-dependent fashion of uterine smooth muscle, small intestine and thoracic aortae in a similar pattern to PGE_2 derivative. Oxytocin also caused uterine strip contraction but had no effect on small intestine. Acetylcholine caused uterine and small intestine contraction in a different manner from that obtained with Ardisiacrispin B. However, the presence of indomethacin did not alter the DR-curve to Ardisiacrispin B of uterine smooth muscle. These results indicate that Ardisiacrispin B exerts a prostaglandin E_2-like effect which may act at the prostaglandin E_2-receptor but not by stimulation or enhancement of prostaglandin synthesis.

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Coraux médicaux

Pascale Joannot

Aquarium, 3, route de l'Aquarium, BP525 Nouméa, Nouvelle Calédonie

Le squelette de certains madrépores est utilisé comme biomatériau en chirurgie osseuse. La formation du squelette de corail semble proche de l'ostéogenèse et aucun rejet n'est constaté lors de l'implantation de ce matériau.

La société INOTEB, détentrice de la license exclusive du corail médical, se fournit essentiellement en Nouvelle-Calédonie.

Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

Pharmacological activities of some plants of family Zingiberaceae

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Division of Pharmacology and Toxicology of Natural products, Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50002, Thailand

Plants of family Zingiberaceae are widely used in traditional medicine, especially of South East Asian countries, and quite a number of studies on their pharmacological properties have been reported. The presentation will be dealt with research works on anti-inflammatory, bronchodilatator and uterine stimulant/relaxant activities of crude extracts of some zingiberaceous plants, and compounds isolated from *Zingiber cassumunar* Roxb. and *Boesenbergia pandurata* and also their analogues.

Third Pacific-Asia Symposium on biologically active natural products, Noumea, New Caledonia, August 26-30th, 1991

Phloeodictine and Thiophloeodictine, novel antimicrobial and cytotoxic guanidine alkaloids from the New Caledonian sponge *Phloeodictyon* sp.

Elly Kourany-Lefoll, T. Sévenet, D. Guénard, A. Montagnac, C. Debitus*, E. Guittet et M. Païs.

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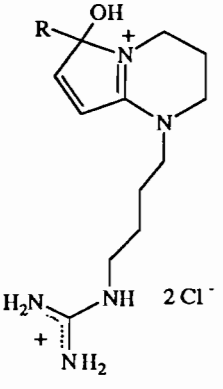
**Centre Orstom, BP A5, Nouméa, Nouvelle-Calédonie.*

In the course of our screening for novel bioactive agents from New Caledonian marine organisms, we have isolated from an undescribed species of the deep sponge *Phloeodictyon* (family Nepheliospongiidae, order Nepheliospongida) two guanidine alkaloids designated as Phloeodictine and Thiophloeodictine. These compounds possess an unprecedented bicyclic skeleton with both a lipophilic alkyl chain and one or two hydrophilic side chains with terminal guanidines.

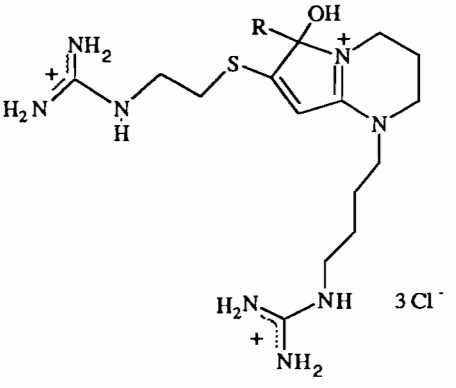
The bioassay-guided isolation of these polar alkaloids from the methanolic extract of the lyophilized sponge involved several separation techniques such as Amberlite XAD-7, reverse phase C_{18} medium pressure column chromatography and ion-pair RP HPLC.

The structures were determined by extensive spectroscopic analysis especially one and two dimensional NMR including HETCOR, DFQ-COSY, LR ^{1}H-^{1}H COSY, HOHAHA, reverse long range ^{13}C-^{1}H and ROESY experiments.

Both compounds showed antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Staphylococcus aureus* and are moderate cytotoxic agents against KB cells.



Phloeodictine



Thiophloeodictine

Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

Chemical and some pharmacological studies of Malaysian medicinal plants

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For the past several years we have been involved in the study of Malaysian plants with particular emphasis on those having traditional medicinal values. A study on the alkaloids of *Alseodaphne perakensis* resulted in the isolation of sebiferine, sebiferine N-oxyde and a non-alkaloidal component 7-hydroxy-2,3,6-trimethoxyphenanthrene. The major alkaloid, sebiferine, was found to be uterine stimulant, hypotensive and having analgesic properties. Poly-pyrroloindolinic alkaloids were isolated from *Psychotria rostrata* with (+)-chimonanthine, (-)-calycanthine and calycosidine were identified as the minor alkaloids. The major tetrameric pyrroindoline was identified as quadrigemine-B and was shown to be cytotoxic. The study on *Lindera pipericarpa* resulted in the isolation of thaliporphine, isocorydine and norisocorydine while *Breynia coronata* gave securinine as the major alkaloid. A tuber of *Cyclea laxiflora*, a root used by the locals for treating sinuses and fever was found to contain mainly dicentrine and the bark of *Orophea polycarpa* contained 5,7,4'-trimethoxyflavanone and 2'-hydroxy-4',6',4-trimethoxychalcone.

A satellite chemical study of natural products have also resulted in an interesting observation. Acid treatment of N-methyl-2,3,6,8-tetramethoxymorphinandien-7-ol gave ring D rearrangement product (and thought to be the biogenetic pathway of protostephanine), whereas similar treatment of the analogous N-methyl-2,3,6-trimethoxymorphinandien-7-ol gave OH-substitution as the major products. This observation thus showed that such rearrangement reaction may proceed in concerted manner rather than through the formation of carbonium ion intermediate.

Third Pacific-Asia Symposium on biologically active natural products, Noumea, New Caledonia, August 26-30th, 1991

Bactericidal oils of *Melaleuca* . A chemical reinvestigation of the essential oil of *M. alternifolia* Cheel (Myrtaceae).

Erich V. Lassak

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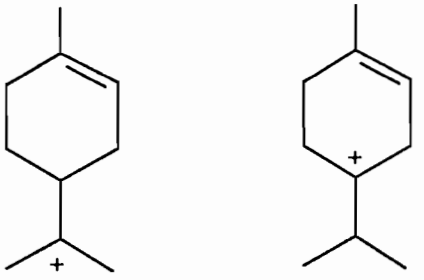
Earlier work by Penfold et al. (1) on the volatile leaf oils of *Melaleuca alternifolia* suggested the existence of three chemical forms characterized by different 1, 8-cineole contents. Of the three forms only the so-called 'type' oil, rich in terpinen-4-ol, has achieved commercial importance as a flavour additive and, more importantly, as a powerful bacteriostatic and bactericidal agent (2-4). Oils used in the present study have been obtained by steam-distillation of a large number of single tree samples from a homogeneous natural population of *M. alternifolia* from coastal northern New South Wales (Australia). Analyses were carried out using capillary GLC and confirmed by MS.

By considering two groups of biogenetically closely related monoterpenoids derived from the precursor carbonium ions (A) and (B) respectively it can be shown that *M. alternifolia* exists in only two and not three chemical forms. These correspond to the two extreme cases where each of these two pathways operates at a virtually total exclusion of the other. The main *M. alternifolia* oil constituents derived from (A) are limonene, 1,8-cineole and α-terpineol, whilst (B) gives rise to α-terpinene, γ-terpinene and terpinen-4-ol.



(A) (B)

Melaleuca dissitiflora (5), *M. linariifolia* and *M. uncinata* also exist in a 1,8-cineole form and a terpinen-4-ol rich form. The latter exhibit bactericidal properties owing to their high terpinen-4-ol content.

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Sapogenine and saponins from two *Albizia* species from New-Caledonia

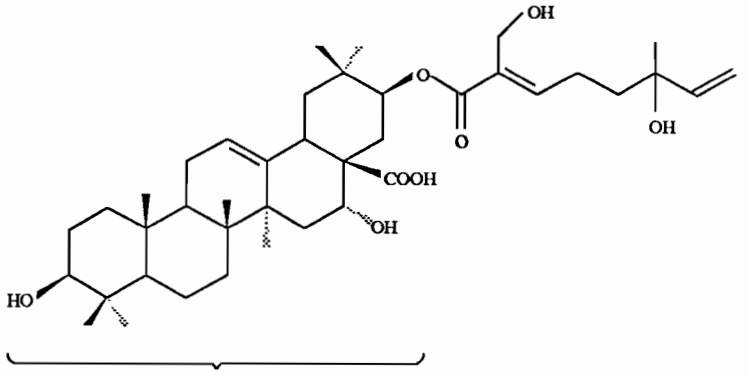
L. Vilela Alegrio, Catherine Lavaud, G. Massiot and L. Le Men-Olivier

Laboratoire de Pharmacognosie, Faculté de Pharmacie, 51, rue Cognacq-Jay, 51096 Reims Cedex, France

Albizia are leguminous plants (Mimosaceae) distributed in tropical and subtropical areas. Their extracts display strong biological activities such as cytotoxic, antiparasitic, immunostimulant. Beside flavonoids and alkaloids, bark and flowers of these plants contain important quantities of saponins. *Albizia granulosa* and *A. streptocarpa* from New Caledonia contain 1.5 and 0.5% of saponins respectively.

Structure of saponins from *Albizia* are usually difficult to solve because they contain three elements which one must sequence : a triterpene, sugars and acids. These latter acids are often labile and their structures are not commonly encountered in natural products.

Using a combination of 1D and 2D NMR, structures of sapogenins, prosapogenins and saponins from these plants will be described.



acacic acid

Girodazole : "from the lagoon of Noumea to cancer patients"

François Lavelle, A. Curaudeau, M. Bayssas, A. Ahond*, C. Poupat*, J. Pusset*, D. Laurent** and P. Potier*

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Girodazole ("girolline", RP 49532A, NSC 627434) is an antitumor agent isolated from the sponge *Pseudaxynissa cantharella* collected in New Caledonia (C.R. Acad. Sci. Paris 307 (II) 145-148, 1988). Girodazole (3-amino-1-[4-(2 amino-1 H-imidazolyl)]-2 chloro-1-propanol, 2HCl) has a unique chemical structure different from those of all the other anticancer drugs and of new compounds in clinical trials.

Girodazole is active *in vivo* on several grafted murine tumors used for preclinical evaluation (P388 leukemia, L1210 leukemia, M5076 histiocytosarcoma, MA/16C mammary adenocarcinoma). In addition, Girodazole retains activity on P388/DOX, a subline of P388 leukemia resistant to anthracyclines and vinca alkaloids.

Antitumor properties of Girodazole are due to protein synthesis inhibition (elongation / termination).

All these innovative properties prompted us to purify large quantities of Girodazole for preclinical, toxicological studies and clinical trials. Several toxic effects were detected in dogs and rodents. However none of them precluded administration in cancer patients.

Phase I clinical studies were done in three european Institutions. different schedules of i.v. administration were used during these tolerance studies. The starting dose was equal to $2mg/m^2$. The dose escalation was interrupted at $15mg/m^2$ by a severe and delayed hypotension uncontroled by antidotes. The plasmatic concentrations of Girodazole obtained in patients at the maximal tolerated dose of $15mg/m^2$ are far below those which preclinical activities were observed in animals. The maximal tolerated dose of $15mg/m^2$ is considered to be too low for expecting clinical responses during phase II clinical trials. Consequently, clinical development has due to be stopped.

Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

La ciguatera en Polynésie Française

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Institut Territorial de Recherches Médicales Louis Malardé, BP30, Papeete, Tahiti, Polynésie Française

La ciguatera, cette intoxication alimentaire d'un type particulier provoquée par la consommation de poissons frais vivant en milieu corallien et appartenant à des espèces habituellement comestibles, est présente en Polynésie Française à raison de 800 cas officiellement déclarés chaque année.

Connue depuis des siècles, son origine a été associée à la prolifération dans certaines conditions écologiques d'une algue microscopique, le dinoflagellé toxique *Gambierdiscus toxicus* Adachi et Fuyuko, et à la transmission de la ciguatoxicité aux poissons herbivores et à leurs prédateurs carnivores ichtyophages.

Deux groupes de toxines sont impliqués dans l'intoxication. L'un est constitué par la maitotoxine, hydrosoluble, et ses analogues éventuels; l'autre comprend la ciguatoxine, liposoluble, et les toxines qui lui sont apparentées.

Une vingtaine de ciguatoxines a été isolée à l'état pur; deux seulement ont été complètement caractérisées par leur structure moléculaire. Codées CTX-1B et CTX-4B, elles ont fait l'objet d'expérimentations toxicologiques et pharmacologiques. CTX-4B, isolée de *Gambierdiscus toxicus* sauvages a une dose létale 50 en toxicité aigüe chez la souris par voie intrapéritonéale, trente fois supérieure à celle de CTX-1B, isolée de foie de poissons carnivores. Toutes deux modifient le courant sodique des membranes excitables, mais CTX-1B est 20 fois plus active que CTX-4B.

Le problème de la détection des ciguatoxines dans les muscles de poissons herbivores et carnivores à une concentration de l'ordre de quelques nanogrammes pour 100 grammes, est en cours d'étude selon plusieurs approches : biologiques, chimiques et immunochimiques.

Par ailleurs, des essais de production de ciguatoxines *in vitro* par culture du dinoflagellé *G. toxicus* au laboratoire ont été réalisés. Les premiers résultats seront présentés.

Third Pacific-Asia Symposium on biologically active natural products, Noumea, New Caledonia, August 26-30th, 1991

Studies on medicine components of *Periplaneta australasiae* : ABO anti-inflammation component

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Cockroach, belonged to Blattidae, is a kind of Chinese folk remedies. According to the reports, it has the functions of anti-extravasated blood, eliminated accumulate, subsidence of a swelling and detoxication, treating pharyngitis, tonsillitis, digestive disorders of children, snake and sting bites, etc...

Cockroach is a kind of pests having great vitality. Biochemical studies have been done before. Protein, peptide, enzymes, blood sugar, carbohydrates, aminoacids, hormone, phenols, fat and its derivatives have been isolated from it. Also it is noticeable that perilanone A and B have been isolated. But as a medicine, it has not been reported up to now.

We have isolated anti-inflammation activity component ABO from *P. australasiae* and worked out a proper procedure.

P. australasiae powder is extracted with aqueous EtOH. The concentrated extractive was dissolved in water. The aqueous liquid was isolated by active charcoal column chromatography. The eluent then was exchanged by Dowex 50. The pharmaceutical activity was tested by Hunan Institute of Medicinal Industry of China with ear of mouse and volume method's anti-inflammation model. The result shows that it has remarkable anti-inflammation effect. Silica gel column chromatography of the residue afforded ABO. ABO has notable anti-inflammation effect which was determined by Yunnan Institute of Drug Control. Qualitative and quantitative analysis prove ABO is a mixture of amino acids.

We have isolated 17 amino acids which necessary for human body from *P. australasiae* total contents reach above 11%.

Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

French national program on "harmful marine algal blooms"

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From the recent available literature, there is provocative evidence of recent significant increase in algal biomass and production, as well as changes in community structure and species distribution in some inshore waters worldwide. It also seems that blooms of toxin-producing species are particularly more frequent. As far as french coastal waters are concerned, three main species have recently caused casualties to shellfish farming or have appeared to be potentially harmful for man health : *Alexandrium minutum* (PSP toxin producer), *Gyrodinium* cf. *aureolum* (hemolysin producer) and *Dinophysis* spp. (DSP Producers). Few other species which have been reported elsewhere to be harmful are present but harmless at the moment : *Chrysochromulina polylepis, Dictyocha* sp., *Prorocentrum minimum.*

In 1989, a national program has been established under the auspices of the Ministeries of Environment, Research and Technology and the Sea, and two national research agencies : CNRS and IFREMER, to stimulate fundamental research on physiological algal capabilities and environmental conditions which lead to toxic blooms (Fig. 1). Multilaboratory cooperative research is greatly encouraged. Integral laboratory and field approaches are recommanded with special attention to time series. Toxin structures and effects are not taken into account; they pertain to a parallel national program : "Phycotoxin".

Within 1989-1990, 13 projects have been funded; one is dedicated to *A. minutum*, five to *Dinophysis* spp., four to *Gymnodinium nagasakiense* (alias *Gyrodinium aureolum*), two to *Prorocentrum micans* and one to *Phaeocystis pouchetii*, and one to all potentially harmful species (e.g. *Chattonella, Olisthodiscus,* etc...). A summary of all available information pertaining to these species has also been done (1). For 1991-92, *P. minutum* and *P. pouchetii* have been withdrew, and possible role of bacteria in toxin production considered as a priority; twelve projects are financially supported. Participants belong mostly to CNRS, IFREMER and University laboratories.

Significant results have been already obtained with the three key species. They are summarized in MAESTRINI *et al* (1991) (2). (i) Research done in the vicinity of the shellfish area of Marennes-Oléron and baie de l'Aiguillon (Charente-Maritime, Vendée) by author's team brings the evidence that *Dinophysis* spp. increases in cell density are associated with warm and stratified waters and occur first in offshore areas, (ii) anthropic nutrient enrichment does not favour *Dinophysis* growth, (iii) *Dinophysis* growth cannot at the moment be related to any typical algal nutrients.

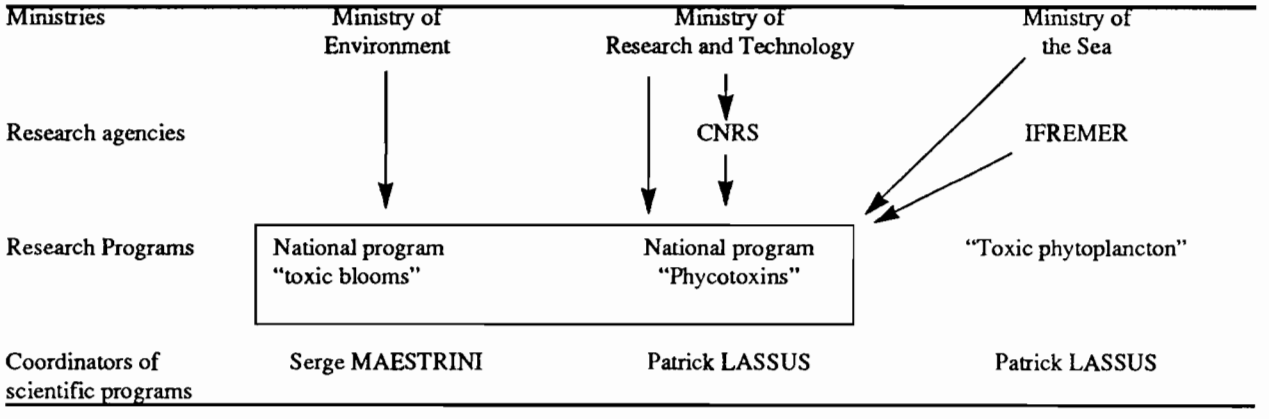

Figure 1: Relationship web between french state departments (Ministries), state research agencies and interlaboratory scientific programs. CNRS; "Centre national de la Recherche scientifique"; IFREMER: "Institut français de Recherche pour l'Exploitation de la Mer". Arrows indicate sources of fundings.

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Third Pacific-Asia Symposium on biologically active natural products, Noumea, New Caledonia, August 26-30th, 1991

Twenty years of chemical investigations on *Alstonia* species from New Caledonia

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The *Alstonia* from New Caledonia form an homogeneous group of 14 species, which were examined from a botanical standpoint by Boiteau, Allorge and Sévenet in1977.

The chemical studies of these species were conducted in Gif, Reims and Chatenay over a twenty year period. Nine of these species, including some varieties, were examined in Reims and the purpose of this paper is to discuss the evolution of phytochemical investigations in the last two decades.

About one hundred different alkaloids have been isolated during these studies, a vast majority being "monomeric" indole alkaloids. Dimeric indole alkaloids are more rare and their structural elucidation is considered "state of the art" in the domain. A key alkaloid from *Alstonia* is cabucraline and the problems debated during its structural elucidation will be discussed along with the solutions put forward in the early seventies. Resolution of this structure may be fully automated now and the problem solved in a matter of hours. Cabucraline is part of more than 10 dimers.

Curiously, *Alstonia undulata* was selected early for chemical investigations and it was soon found that its alkaloid mixture was an intricate combination of complex and unstable dimeric alkaloids. Several assaults were launched against the alkaloids and each attack yielded some information but it was not until the early nineties that reasonable structures were put forward for the so-called "purple" alkaloids.

The tools of Phytochemistry have changed over the years but also the purposes of this research. Was it necessary to spend all this time and money to study *Alstonia* from New Caledonia? These points will be discussed.

Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

Animal toxins : structural economy but functional prodigality.

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Animal venoms are complex mixtures of proteins among which some are potent toxins. The toxins are capable of a wide diversity of biological effects on the neuromuscular junction, autonomic ganglia, central nervous system, heart and plasmatic coagulation system. At these sites, they often block critical receptors, ligand gated ion channels or enzymes, sometimes by competing with an endogeneous molecule. A multiplicity of functionally different toxins has been identified and new toxins are currently discovered every year.

What is the structural basis for the functional variability of animal toxins?

Animal toxins are often small in size with their polypeptide chain comprising less than 120 amino acid residues and several disulphides bridges. Strikingly, the same overall structural architecture is used to exert several different functions. For example, most snake toxins belong to one of three different strutural classes. These are (i) toxins having a "three finger-shaped structure", a group which includes curaremimetic toxins, fasciculins, muscarinic toxins, neuronal toxins, synergistic-type toxins, toxins that block tachykinin receptors, cardio(cyto)toxins and presumably calciceptine, a calcium channel blocker; (ii) toxins folded like phospholipases-A_2 and (iii) toxins folded like trypsin inhibitor. A similar situation is encountered with scorpion toxins. Thus, irrespective of size and function, these toxins share the same 3-D motif which includes a small triple strand of antiparallel ß-sheet linked to a short helix by disulphide bonds. Clearly, analogous structures of an animal toxin can support a variety of biological functions. On the other hand, the same target can be aimed at by toxins having different structures. For example, α-conotoxin GI, a small peptide of 13 amino acid residues produced by a marine snail, competes with a snake curaremimetic toxin, a protein of 60-74 residues, for binding to the nicotinic acetylcholine receptor. These molecules have different architectures but nevertheless, they respectively possess areas that are spatially organized in a similar manner, enabling them to exert comparable functions. Structures of animal toxins are therefore characterized by a remarkable functional adaptability.

Do structurally related toxins have topographically similar functional sites?

To answer this question, we studied curaremimetic toxins and cytotoxins from cobra venoms which, despite their functional differences, share highly similar architectures. Their polypeptide chain is folded into three adjacent loops (I, II and III) that are rich in ß-sheet and protrude from a little core containing four invariant disulphides. We identifed the two "toxic" sites using a set of genetic and/or chemical mutants of either toxins. Data showed that the "curaremimetic site" is essentially located on loops II and III whereas the "cytotoxic site" is mostly located on loop I and perhaps, at the base of loop II. The two sites are clearly topographically distinct. Therefore, no structurally specific area seems to be responsible for the functional diversity of snake toxins, in contrast to other proteins like, for example, immunoglobulins.

In conclusion, architectures of animal toxins are attractive proteic templates for the design of pharmacological functions.

Third Pacific-Asia Symposium on biologically active natural products, Noumea, New Caledonia, August 26-30th, 1991

Quenchers against singlet oxygen in symbiotic bacteria of marine sponges

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A number of biologially active substances have been isolated from marine sponges. Aromatic carotenoids, such as renierate, isorenieratene, renierapurpurine and others, have only been purified in marine sponges and they showed a strong quenching activities against singlet oxygen. Recently, it has been presumed that these bioactive compounds are not originated in sponges themselves, but in symbiotic microorganisms. On the basis of these view point, we started to screen biologically active compounds from symbiotic bacteria of marine sponges, using the assay system for quenchers against singlet oxygen [1].

Zeaxanthin (4,4'-dihydroxy-ß,ß-carotene), one of the major carotenoids in marine organisms, was first isolated from symbiotic bacteria of a marine sponge, *Halichondria okadai*. The other symbionts of the sponge were found to produce two novel carotenoids, okadaxanthin [2,2'-bis (4-hydroxy-2-methyl-2-butenyl)- ε,ε-carotene] and halixanthin [(3R)-1',2'-dihydro-3', 4'-didehydro-3,1'-dihydroxy-ß,ψ-caroten-4-one], which show strong quenching activities.

Reference

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Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

Marine natural products : chemical constituents from New Caledonian deep-water species

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During our ongoing program of searching for new bioactive molecules from new-caledonian marine invertebrates, the opportunities occured recently to examine a "living fossil" crinoid *Gymnocrinus richeri*, discovered by B. Richer de Forges at 520m depth. *In vivo* this crinoid is saffron yellow with the stalk darker and tentacles dark yellow-green inside. A few minutes after collecting, outside the water, it turns readily dark-green. The green pigments, extractable with methanol, turned violet on very mild acidification.

In this communication the structure of five violet pigments, which constitute a novel group of brominated phenanthroperylenequinones, will be discussed. These pigments have interesting stereochemical features, *i.e.* the axial chirality generated by the phenanthroperylenequinone system forced into a non planar helical shape. The assignment of the stereochemistry based on CD, NMR data and correlation with natural occuring perylenequinones will be presented. There is also considered the possible relationship between the violet pigments and the native yellow and green ones.

A second "living fossil" organism from New Caledonia which we had the opportunity to examine is the starfish species *Tremaster novae caledoniae* collected at 530m depth off Nouméa. This organism contains a group of unusual steroids in which one hydroxyl group is sulphated, one is acetylated and a third one is esterified with glucose-1-phosphate.

The results of the chemical investigation of the sponge *Jereicopsis graphidiophora* (new genus) and *Erylus* sp. collected at ca. 500m depth off Nouméa, will be also presented. While the 3ß- hydroxy steroids were totally absent, the extracts of *J. graphidiophora* contain unique 3ß- hydroxy steroids. Two of them combine the unique 3ß- methoxyl group with a rare secostructure.

The polar extracts of *Erylus* sp. contain two terpenoid oligoglycosides. Sequential analysis of the oligosaccharide portions was achieved by modern 2D-NMR techniques.

Third Pacific-Asia Symposium on biologically active natural products, Noumea, New Caledonia, August 26-30th, 1991

Coral reef ascidians of New-Caledonia

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Many pharmacologically active compounds have been recently described in marine animals. Tunicates are an abundant source of interesting products. Most of them show antibacterial, antitumor or antiviral activities, others being rarely tested. A complete investigation of toxins in Ascidians is difficult to set up for several reasons:

1) Ascidians are difficult to collect, and even to recognize; they often live in crevices or under stones in thin encrusting sheets.

2) They quickly die out of water, the tissues decompose and the organic compounds are modified.

3) Identification is difficult : it always requires fixation, dissection, staining and observation under a microscope; moreover, the animals have to be mature with both male and female gonads and incubated embryos.

Another difficulty is the very small number of taxonomists and the absence of identification manuals for Tunicates in spite of the fact that these animals are present in all seas, at all latitudes from intertidal shores to abyssal depths.

An ambitious program of ORSTOM in New-Caledonia is to make an inventory, and conduct an ecological survey of the marine fauna of the lagoon where possible species are being photographed underwater to show their natural colours and habitat. For ascidians, the commonest and most spectacular animals are presented in a richly illustrated book. The purpose is to motivate naturalists and scuba divers to take some interest in such strange immobile animals, the ancestors of vertebrates, and to help in recognizing them underwater. But we have tried to go further by explaining their anatomy and ecology, and their parasites and symbionts. The economic interest in aquaculture and fouling is also discussed.

No doubt biochemists will be excited to investigate more ascidians in search of drugs, when they see how beautiful and varied they are in life.

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Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

Activité antileishmanienne et antimicrobienne des alcaloïdes de *Peschiera van heurkii*

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Dans le cadre de l'étude des plantes antiparasitaires de Bolivie actuellement effectuée[1], nous présentons les résultats obtenus avec *Peschiera van heurkii* L. Allorge, Apocynaceae, un arbuste abondant dans les zones tropicales basses de Bolivie.

Les feuilles et les écorces de tiges sont riches en alcaloïdes : 12 et 36g/kg respectivement. Du totum alcaloïdique ont été isolés 20 alcaloïdes, en majorité des monomères du type Vobasine Affimisine, et des dimères tels que Conodurine, Conoduramine et Gabunine. Les structures ont été établies par comparaison avec des échantillons de référence et par RMN 1D et 2D.

L'activité antimicrobienne de tous les alcaloïdes a été mesurée sur les souches suivantes: *S. aureus, E. coli, P. aeruginosa, M. smegmatis, B. subtilis* (technique de diffusion sur agar-agar). Les plus actifs sont la Conodurine et la Conoduramine.

Le totum alcaloïdique s'est avéré toxique pour les *Leishmania* du Nouveau Monde : *L. mexicana amazonensis* et *L. brasiliensis brasiliensis*. Les alcaloïdes les plus actifs sont la Gabunine et la Conodurine.

[1] Convention IBBA-ORSTOM-UMSS Cochabamba : Etude des Plantes antiparasitaires de la Région Cochabamba-Chapare.

Third Pacific-Asia Symposium on biologically active natural products, Noumea, New Caledonia, August 26-30th, 1991

Pateamine : a case study for the isolation of compounds with biological activity

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The most critical decision to be made in the search of biologically active metabolites is the actual choice of plant or animal species to commence work on. To assist in this decision making a protocol for the "CHEMICAL SCREENING" of an extract has been developed. The chemical screening establishes the physical properties of the active component(s) and gives a clear indication as to the most efficient pathway to the isolation of the active compounds. If this was not advantage enough chemical screening is also particularly useful as an aid in the dereplication of extracts.

The utility of chemical screening will be illustrated using pateamine as the prime example. Pateamine, a macrocyclic diamine, is a potent, but apparently selective, cytotoxin isolated from a New Zealand *Mycale* sp. Recent work on the structure, chemistry, biology and molecular modelling of pateamine will be presented.

MarinLit: a marine literature database

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A vital component in any research programme is awareness of the chemical literature both past and current. To assist our endeavours in marine natural products chemistry MarinLit, a marine literature database, was established in 1984. MarinLit was designed around Apple computers and the current version uses FoxBase + /Mac to establish the relational data base. MarinLit requires 1Mb RAM and 7Mb of disc space and is operative on all Macs.

In constructing this database, the focus has been on creating a tool useful at the **outset** of an investigation. The emphasis therefore is on taxonomic and biological data-information not readily available from CAS. The various other features of MarinLit such as molecular weight and molecular formula searching, as well as the extensive use of keywords (>300) as descriptors will become apparent on using the database. MarinLit will be available for use during the poster session.

Autotomy promoting factor (APF) in south west Pacific sea stars

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Autotomy promoting factor (APF) is a peptide or peptide-like substance found in the body fluide derived from scaled sea stars. APF was first detected in the North Pacific sea star, *Pycnopodia helianthoides*. When injected into individuals of the same species, APF elicits arm autotomy and a generalised softening of the connective tissues of the body wall.

In this study we report the detection and isolation of a similar factor from three unrelated New Zealand sea stars, both fissiparous and non-fissiparous, *Sclerasterias mollis, Coscinasterias calamaria* and *Astrostole scabra*. Extracts prepared from all three species were bioassayed in *S. mollis*, resulting in complete autotomy of 1-4 arms over a period of 15-120 mins. Species cross reactivity was observed in all cases. The autotomy process is similar to that reported for the North Pacific species, *Pycnopodia helianthoides*, but occurs on a slower time scale.

Crude and partially purified APF from the above sources is a complex mixture of peptides, proteins and other molecules. Initial purification was carried out using the ultrafiltration and gel permeation chromatography, followed by reverse phase HPLC. Initial partial purification studies indicate that APF in these species is similar to that reported by Mladenov *et al.* (1989) with a molecular weight in the region 1200-1400. This is consistent with a peptide of 10-12 residues in length.

Autoclaving of the crude extract results in a 2-3 X increase in the rate of autotomy in bioassayed animals, though whether this results from inactivation of proteases present in the crude extract, activation of an inactive precursor, or release of a previously sequesterd form, remains undetermined.

Final purification studies and peptide sequencing of APF are currently being undertaken.

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Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

Problèmes posés par la détection de la ciguatoxine

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Jusqu'à présent, les toxines localisées dans la chair de poisson ne peuvent être détectées en routine que par des tests biologiques sur animaux sensibles : mangoustes, chats, poulets et moustiques. La très faible corrélation existant entre les différents tests biologiques (Kimura et coll., 1982, Bagnis et coll., 1985) met l'accent sur la nécessité d'un dosage plus chimique ou plus sélectif des toxines présentes dans les échantillons testés. Cependant, quelque soit le procédé, physico-chimique ou immunochimique, à utiliser, la difficulté majeure réside dans l'obtention d'un seuil de sensibilité suffisamment bas pour en permettre la détection de très faibles taux (de l'ordre de quelques ppb) dans les échantillons. Cette difficulté ne peut être, pour l'instant, résolue que par des étapes d'extraction en solvants organiques suivies d'enrichissement et de purification chromatographiques efficaces mais contraignantes. C'est ainsi que Legrand et coll. (1990) ont mis en évidence l'existence d'une des familles de CTX.

La première tentative de dosage physico-chimique de la ciguatoxine (CTX) a été réalisée en chromatographie liquide à haute pression (HPLC) en utilisant un dérivé fluorescent de la CTX obtenu par synthèse. Ce dosage combinant des techniques chromatographiques et fluorimétriques nécessite encore une étape d'extraction de la CTX et ne permet, pour l'instant, qu'un seuil de détection de l'ordre du ng/injection (Legrand et coll., 1990, Fukui et coll., 1991). En raison de la lourdeur actuelle de ces manipulations, une voie d'approche possible de ce problème consiste en une détection immunochimique qui pourrait aboutir à un procédé non seulement simple, rapide et fiable mais aussi peu coûteux et transposable à grande échelle. Sur ce point, les travaux du Pr Hokama d'Hawaï, quoique prometteurs, ne sont pas exempts de critiques : les immunogènes utilisés pour l'obtention d'anticorps polyclonaux (Ac) et monoclonaux (Acm) ne sont pas caractérisés ; de plus, dans les différents tests proposés, la distinction entre poissons sains et ciguatériques repose sur de très faibles écarts de mesure.

Le couplage d'un haptène lipidique à une protéine porteuse pose déja en lui-même le problème de la solubilité des deux partenaires dans une phase semi-organique, mais lorsqu'il s'agit de manipulations sur d'infimes quantités de produits, l'on doit s'attacher en outre au rendement des réactions. Dans notre laboratoire, compte tenu des faibles quantités de CTX pure dont nous disposons, les essais préliminaires de préparation d'immunogènes stables et parfaitement définis ont été tout d'abord réalisés sur la momensine (antibiotique polyéther de faible valeur commerciale) en utilisant dans toutes les étapes quelques centaines de mg puis de µg.

Plus récemment, ces procédés ont été appliqués à la PbTX-3, membre de la famille des brévétoxines (BTX), de structure chimique très proche des CTX et depuis peu commercialisées. Des Acm anti-momensine de haute affinité (K_D = 1,5 x 10^{-9}) ont été obtenus, montrant qu'il était possible d'obtenir par cette voie des Acm anti-polyéther.

Les travaux sur la PbTX-3 se poursuivent et dans l'optique d'un rendement optimal, plusieurs procédés seront encore évalués quant à leur efficacité de couplage. Du fait de la ressemblance structurale entre les CTX et les BTX, nous espérons obtenir des Acm reconnaissant parfaitement la CTX de référence extraite du foie de murène, mais aussi les autres toxines apparentées. Cependant les autres possibilités de détections basées sur le pouvoir cytotoxique sur cellules cible ou l'utilisation de nouveaux bio-récepteurs ne doivent pas être pour autant abandonnées. Sur ces deux derniers thèmes, des travaux sont en cours à l'ITRMLM pour les cellules cibles et à l'Institut Pasteur de Paris pour les bio-récepteurs.

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Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

Alteration of cellular events involved in the first cleavage of sea urchin egg by two marine toxins : "Maitotoxin" and "Crassolide"

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In this study the sea urchin egg model was used to investigate the effects of **Maitotoxin (MTX)** synthetized by the epiphytic dinoflagellate ***Gambierdiscus toxicus*** and by **crassolide** a diterpene isolated from the Indonesian soft coral ***Lobophytum crassum*** .

Sea urchin eggs are convenient to use for bioassays since it is easy to obtain cells dividing synchronously. In addition, the schedule of ionic events along the cell cycle is one of the best known showing the same succession as mammal cells culture.

Maitotoxin is known to stimulate a large spectrum of calcium dependent physiological processes; it is a potent activator of calcium influx in a large variety of cells. Fertilization and cleavage of sea urchin eggs were inhibited in a dose dependent manner by MTX. This toxin increased the permeability to Ca^{2+} of both plasma and intracellular membranes and modified K^+ and Na^+ distribution in the female gametes. Toxin induced changes in ion permeabilities were observed at a concentration much higher than those inhibiting fertilization and did not evolved rapidly. Therefore, the blocking of fertilization which occurred at low MTX concentrations and appeared in a short time is probably not due to ion transport perturbation : a modification of the unfertilized egg plasma membrane by the hydrophilic toxin could be involved.

Crassolide inhibited the cell cleavage of sea urchin eggs without affecting fertilization. The effect was observed at concentrations above 2×10^{-5}M in egg suspensions. Addition of crassolide between 5 to 40 minutes after fertilization totally blocked the first cleavage. When added between 50 to 60 min. post fertilization, crassolide produced polynucleated cells in embryos. Moreover, it did not affect egg permeability to Na+ and Ca^{2+} but caused a 0.2 unit increase of intracellular pH of fertilized eggs coupled with a proton efflux. Crassolide which affects neither Ca^{2+} influx nor Ca^{2+} permeability of reticular store could be used as negative control when analysing calcium changes in short-term toxicological studies. A possible relationship between pH increase and cell cleavage needs further investigations.

A set of preliminary experiments concerning the inhibitory effect of crassolide on the proliferation of cultured fibroblasts (BHK 21/C3) shows that concentrations comparable to these used on sea urchin eggs (1.7µg/ml), blocks 50% of fibroblast growth.

Third Pacific-Asia Symposium on biologically active natural products, Noumea, New Caledonia, August 26-30th, 1991

Gambierdiscus toxicus and ciguatera

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In 1977, Yasumoto et al. found ciguatoxin-like and maitotoxin-like toxins in samples of algae containing large amounts of ***Gambierdicus toxicus.*** Up to this date this benthic dinoflagellate is considered as the main toxin producer yielding to ciguateric fishes via the marine food chain.

We have conducted a study of *G. toxicus* populations at **Saint Barthélémy** from 1985 to 1987. Because this **Caribbean island** is highly affected by **ciguatera fish poisoning**, it is a good model for studying the link between *G. toxicus* toxicity and poisoning incidence.

We have found that *G. toxicus* was located all around St Barthélémy at low cell densities from 5 to 500 cells per gram of wet macrophytes, with perhaps a maximum of abundance in Spring. Photonic and electronic microscopy showed no difference in regard to **Pacific specimens.**

Using the mouse bioassay (intraperitoneal injection), toxicities of crude methanolic extracts (mostly MTX) were found to be very close (1000 cells of *G. toxicus* are necessary to kill a 20g mouse within 24 h.), whereas after separation on silicagel column, the toxicity of the chloroform-methanol (9:1) fraction (CTX-like) was higher in the caribbean sample (around 1% instead of 0,1%). This difference may be due either to a lost of toxicity of the **Gambier** strain during the culture period or to the presence of different bacterial communities. Research is in progress to elucidate this point.

Our study could be summarized by : **- high incidence of ciguatera in both Gambier islands and St Barthélémy but -large cell density, low CTX-like toxicity in the former, -low cell density, higher CTX-like toxicity in the latter. Therefore both toxicity and abundance of *G. toxicus* should be taken into account for evaluating the role of *G. toxicus* in ciguateric areas.**

Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

Antiprotozoal agents from higher plants

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A number of major tropical diseases, including malaria, leishmaniasis, trypanosomiasis and amoebiasis, are caused by protozoa. *Cinchona* bark, used for the treatment of malarial fevers, has yielded quinine which has been used for many years as a main clinical drug against malaria. Similarly, emetine isolated from the roots of *Cephaelis* species has proved to be clinically effective against amoebiasis. Of these two drugs it is quinine, in particular, which has served as a template molecule for the development of new synthetic antimalarials. A series of 8-aminoquinolines, e.g. mepacrine and 4-aminoquinolines, e.g. chloroquine, introduced into clinical practice, owe their origin to the natural alkaloid quinine.

Plasmodium falciparum, the cause of human tertiary malaria, has become resistant to chloroquine in many tropical countries and there is an urgent need for new antimalarial drugs. No curative drugs exist for Chagas disease caused by *Trypanosoma cruzi* and which is responsible for the death of millions in S. America. Leishmaniasis occurs as a cutaneous disease in Asia due to infections of *Leishmania tropica* and in S. America due to *L. mexicana*. Visceral forms of the disease are caused by *L. donovani* in both African and Asian countries. The available treatment has severe limitations and all forms of the disease are difficult to treat. Although nitroimidazole drugs such as metronidazole are effective against *Entamoeba histolytica*, the protozoan responsible for amoebiasis, the drugs are not always well tolerated by the patients.

The plant kingdom offers the possibility of new drugs for the treatment of protozoan disease and the discovery of the sesquiterpene endoperoxide artemisinin from *Artemisia annua* as being clinically effective against chloroquine-resistant malaria has, once again, focussed attention on higher plants as sources of antiplasmodial drugs. Many plants are used throughout the world in systems of traditional medicine for the treatment of protozoal diseases and such plants merit detailed scientific investigations.

A number of isoquinoline- (e.g. berberine, bisbenzylisoquinolines), indole- (e.g. ß-carboline, usambarensine, cinchophyllines) and acridone- (e.g. atalaphyllinine) type alkaloids have antiprotozoal activity. Several terpenoids including quassinoids, limonoids, di- and sesqui- terpenes show potent activity as antiprotozoal agents. Other plants constituents with antiprotozoal activities include various flavonoids and naphtoquinones.

A wide range of structural types have already been shown to be effective *in vitro* but more detailed studies of their *in vivo* activities and modes of action need to be undertaken. *In vitro* tests are now available for bioassay guided fractionation of plants extracts and may be used for the evaluation of the efficacy of traditional medicines as well as for new drug molecules.

Third Pacific-Asia Symposium on biologically active natural products, Noumea, New Caledonia, August 26-30th, 1991

Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

Preliminary study of a neocaledonian sponge : *Axinella carteri* Dendy.

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Axinella carteri Dendy sample R 1257 was collected in the lagoon of Noumea on the "Dôme de l'Ilot Canard" at a depth of 6-10m. The water temperature is 25+/-2°C.

This preliminary report concerns the first study of this sponge which had been shown previously by ORSTOM searchers at Noumea to have a cytostatic activity.

After multiple chromatography on both columns and preparative t.l.c. of the acetone extract, it was possible to isolate two groups of compounds which are extremely difficult to separate due to their close chemical nature.

The first group contains as the major constituant 3-ß-hydroxymethyl-A-nor stigmastane. **1** along with its unsaturated homologues Δ-22, **2** and Δ-15, **3**. Similar mixtures have already been reported as present in other sponges (*A. verrucosa, Homaxinella, Acanthella, Phakellia*).

The stereochemistry 3-ß has been confirmed by the NOESY spectra.

The second group contains the eicosenoic esters of **1** and **2**.

We wish to thank the divers of the ORSTOM centre for collecting the material and Prof. C. Lévi of the Museum d'Histoire Naturelle who has kindly identified the sponge.

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Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

Alkaloids from leaves of *Cryptocarya phyllostemon* Kost.

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Among the 350 species of the pantropical genus *Cryptocarya* (Lauraceae, subfamily Lauroideae, tribe *Cryptocaryeae*, subtribe *Cryptocaryniae*), 19 grow in New Caledonia.

The endemic species *C. phyllostemon* Kost is a 28m, high tree with lanceolate, medium sized leaves (2 by 7cm) and small conical, bluish fruits (1.2 by 2.7cm).

A previous study, carried out simultaneously by two different Laboratories on two batches collected in Haute Ouinné Valley (Mts Dzumacs) : Sévenet 644 and PC-NC 219 in 1974 and 1976 respectively led to the isolation of 7 alkaloids from the first batch (whole plant) and 6 from the latter (stem bark). Only 3 alkaloids were common to the two batches : antofine, the main one, phyllocryptine, phyllocryptonine.

The present study describes the chemical composition of another batch (leaves) of *C. phyllostemon* collected in 1968 in Haute Rivière Bleue districts and formerly identified as *C. gracilis* Schlechter. Among the 7 alkaloids isolated, only two have been already discovered in batch Sévenet 644 : antofine and phyllostemine. The others are tylophorinine, tylophorinidine (*Tylophora asthmatica*, Asclepiadaceae), oubacryptine (*C. oubatchensis*) and two new bases. O-methyl-4"- phyllostemine and isotylophorinine.

In conclusion, the minor secondary metabolites of *C. phyllostemon* seem to vary highly depending of the location of the plant, with the main alkaloid, antofine, being always present.

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Synthèse de peptides à activité cytolytique

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De nombreux peptides naturels présentent une activité cytolytique. Le mécanisme qui rend compte de cette activité est variable d'un groupe de toxines à un autre et souvent complexe.

Les structures de ces toxines présentent également une grande variabilité et peuvent comporter une dizaine à quelques centaines d'acides aminés. Pour des peptides de taille comparable, les séquences elles-mêmes sont très variables et le degré d'homologie très faible.

Cependant, il a été observé que des éléments de structures similaires semblent présents dans plusieurs de ces toxines; c'est ainsi qu'un fragment structuré en hélice α est souvent présent. De même un fragment fortement basique, comprenant de 5 à 10 acides aminés, est également présent dans plusieurs peptides.

Nous avons examiné de plus près les séquences de peptides à activité cytolytique et nous avons cherché à construire des peptides répondant à certains critères structuraux afin de leur conférer une activité cytolytique.

La construction de ces peptides et leur synthèse sont présentées sur ce poster.

Possibilités d'utilisation de substances lichéniques comme protecteurs solaires

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L'accroissement mesuré des radiations ultraviolettes à la surface de la terre, dû principalement aux trous de la couche d'ozone, provoque des problèmes de santé chez l'homme tels que l'augmentation progressive des cancers de la peau, donnée par les dernières statistiques.

Les lichens synthétisent et accumulent des concentrations variables de produits du métabolisme secondaire qui répondent à cinq structures de base : les depsides, les depsidones, les diphényléthers, les dibenzofurannes et les acides usniques (Elix et al. 1984). Ces composés absorbent dans la région du spectre solaire des UV A et UV B, qui comprennent les radiations les plus nocives, ceci étant dû à leur meilleure pénétration dans la peau (290 nm à 350 nm). A l'acide usnique, l'un des métabolites les plus fréquents dans les espèces lichéniques, on attribue un rôle filtrant d'une fraction de la radiation UV, protégeant les algues et les cyanobactéries symbiotiques dans les thalles (Lawrey, 1986). D'autres depsides comme l'atranorine, l'acide divaricatique et l'acide gyrophorique, ou depsidones comme la vicanicine, la pannarine et la 1'- chloropannarine absorbent la radiation UV proche de la région visible du spectre, entre 430 nm et 485 nm (Hidalgo et al., 1981).

On a constaté pour l'atranorine, la pannarine et la 1'-chloropannarine une grande stabilité à la lumière. Cette qualité et leurs caractéristiques spectrales, nous permettent de proposer une utilisation possible de ces composés pour l'élaboration de filtres solaires.

On a été informé que quelques métabolites lichéniques produisent des dermatites de contact (Wennersten, 1979). Afin de vérifier la toxicité sur les membranes cellulaires, ainsi que les réactions de photosensibilité aux composés cités ci-dessus, le test de photohémolyse a été réalisé : les résultats montrent que seules la pannarine et la 1'-chloropannarine produisent quelques faibles altérations photohémolytiques (Hidalgo *et al.*, 1989).

Aussi est-il important de remarquer que ces substances, de structure phénolique, montrent des capacités antioxydantes mises en évidence par les essais d'autoxydation d'homogénéisat de cerveau de rat et de l'autoxydation du ß-carotène.

Par leurs propriétés photochimiques et antioxydantes, les substances lichéniques pourraient protéger la peau des mauvaises radiations et des processus oxydatifs.

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Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

Rôle des bactéries associées aux dinoflagellés dans la production de toxines. Application à *Dinophysis sp.* et *Prorocentrum lima.*

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Des travaux récents ont été réalisés sur le rôle des bactéries associées aux dinoflagellés *Alexandrium tomarense* (Kodama et al, 1988, 1989, 1990), *Gymnodinium breve* (Buck et Pierce, 1989) et *Gambierdiscus toxicus* (Tosteson et al, 1989). Ils tendent à montrer que les bactéries seraient impliquées dans la production de toxine, voire qu'elles en seraient les productrices. Aucun travail n'ayant à ce jour été réalisé sur les bactéries associées aux dinoflagellés responsables d'intoxication DSP, nous avons extrapolè ces données à ce type de dinoflagellé afin d'évaluer le rôle des bactéries associées à deux dinoflagellés producteurs de DSP (*Prorocentrum lima* et *Dinophysis* spp.) dans la production de toxines.

Une étude ultrastructurale de ces deux dinoflagellés au MEB a permis de visualiser les très nombreuses bactéries de forme hélicoïdale fixées aux cellules de P. lima, ainsi que le faible nombre de bactéries, de forme classique, fixées sur les cellules de *Dinophysis*.

Une recherche d'acide okadaïque par dosage HPLC dans les bactéries libres (non fixées aux cellules phytoplanctoniques) a été entreprise dans les cultures de *P. lima* d'une part, et à partir d'eau de mer prélevée lors d'une prolifération de *Dinophysis* d'autre part. Les premiers résultats montrent que les bactéries libres associées à *P. lima* contiendraient une faible quantité d'acide okadaïque ; et que la stimulation de la croissance de ces bactéries n'augmente pas la teneur en toxine dans les bactéries libres.

Third Pacific-Asia Symposium on biologically active natural products, Noumea, New Caledonia, August 26-30th, 1991

Cytotoxic bispyrones from *Onchidium sp.*: absolute stereochemistry of onchitriols I and II

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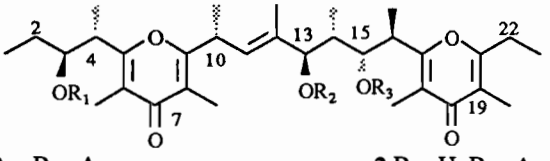
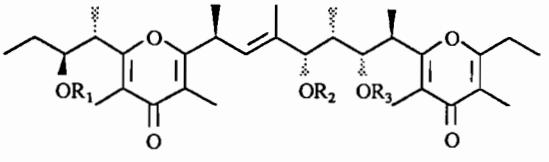
The *Onchidiaceae*, shell-less molluscs inhabiting the rocky intertidal zones of many tropical shorelines, are known to produce cytotoxic pyrone-containing propionate and acetate metabolites. Peroniatriol I and II were isolated by Ireland[1] *et al.* after saponification of the extracts from *Peronia peronii*; elucidation of their structure and relative stereochemistry was based on those of ilikopyrone[2] and corrected by partial synthesis[3].

We now report the isolation, by reverse phase hplc, of cytotoxic extract of *Onchidium* sp. collected in New Caledonia of the metabolites identified as the acetates and propionates **1-4** and **6-9**.

The skeletal structure of **1-4** and **6-9**, and the exact positions of the acetate and propionate groups, were deduced by MS (EI and FAB) and NMR (homonuclear and heteronuclear correlations). Upon saponification in 1% KOH/MeOH at r.t. **1-4** gave onchitriol I and **6-9** gave onchitriol II (**10**). NMR studies of the mono, di, tri (R) and (S) O-methylmandelates, dioxolane formation and reducive ozonolysis showed the absolute stereochemistry of **5** to be 3*S* , 4*R* , 10*R* , 13*R* , 14*R* , 15*R* , 16*R* and that of **10** 3*S* , 4*R* , 10*S* , 13 *S* , 14 *R* , 15*R* , 16*R*.



1 R_1=H, R_2=R_3=Ac
2 R_1=H, R_2=Ac, R_3=Pr
3 R_1=H, R_2=Pr, R_3=Ac
4 R_1=Ac, R_2=R_3=Pr
5 R_1=R_2=R_3=H



6 R_1=H, R_2=H, R_3=Ac
7 R_1=H, R_2=H, R_3=Pr
8 R_1= R_3=Ac, R_2=H
9 R_1= R_2=R_3=Ac
10 R_1=R_2=R_3= H

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Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

Chilean marine organisms : secondary metabolites and its biological activities

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Chile presents a large coast with cold water, where there are many endemic organisms. Our research programs are oriented to bioactive substances from mollusks, sponges and algae. In this opportunity we present the results of the chemical and biological analysis of *Plocamium cartilagineum* (collected in Chilean coast of Antartic Peninsula), *Laurencia claviformis*, *Stypopodium flabelliforme*, *Siphonaria lessoni* and the antarctic sponge *Dendrilla membranosa*. The metabolites isolated were tested against Gram positive and Gram negative bacteria and a yeast. Also, some of them were submitted to an insecticide/acaricide screen and others to a fungicide and herbicide screen.

Acknowledgements : This study was supported by FONDECYT (Grants 1171/89 and 1069/90), DTI (U. de Chile), Instituto Antartico Chileno. PAE, CICY (MEC, España)

Third Pacific-Asia Symposium on biologically active natural products, Noumea, New Caledonia, August 26-30th, 1991

Structural Characterization of modified starch

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Starch, a common constituent of higher plants is a major form in which carbohydrate are stored. Most starches are mixtures of two macromolecular entities : amylose which is a linear polymer of rather low molecular weight (10^3 to 10^4 α-1-4-D-glucopyranosyl residues) and amylopectin composed of same residues and branched, of very high molecular weight. Starch and amylopectin are now currently used in industrial application particularly as thickening agents and food gelifiers. Nevertheless amylopectin whose a schematic modele is given in fig.1, is badly water soluble because the helices of the branches form crystalline domains which are not entirely destroyed by hydratation at least by current solubilization process.

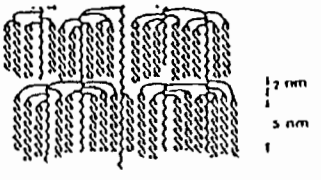
It is the reason why they are often used in a modified form. For instance a serie of products prepared by Roquette SA and called HES are hydrophobically modified amylopectin (see reaction in fig 2) which are more soluble.

We are interested in the understanding of the relations between the molecular weight, branching and local structure) and the thickening properties of such polymers. We have recently performed a systematical study by elastic or quasi elastic light scaterring on different unfractionnated HES and two series of fractions of polydispersity IP: $1.2<IP<1.4$ and molecular weight M_w: $4.10^4<M_w<10^6$. The following scaling laws were found for the molecular weight dependences of the static and dynamic radius of giration and viscosity:

$$R_g = 0.044\ M^{0.44}$$
$$R_H = 0.091\ M^{0.40}$$
$$[n] = 2.34\ 10^{-2}\ M^{0.20}$$

In this range of explored molecular weight, the radius of giration is ranging between 5 to 20 nm so we undergo SAXS measurements to determine also radius of giration and the structure of the graft parameters with the molecular weight. The determinations of these parameters are useful for all the applications.

Fig. 1: schematic model of amylopectin molecule from A-type starch granule



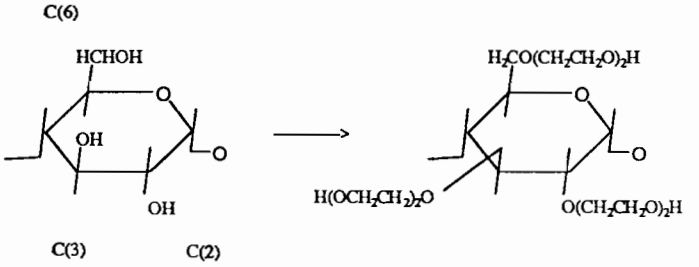


Fig. 2

Activité antipaludique de la cedronine isolée de *Simaba cedron* Planchon

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Les quassinoïdes possèdent de nombreuses propriétés biologiques : antinéoplasiques, antivirales, antiamibiennes, antipaludiques. Les quassinoïdes ayant la plus forte activité antipaludique et antivirale, comme la Brucéantine, le Simalikalactone D, le Sergeolide, ont un squelette carboné en C20 et montrent aussi une activité significative *in vivo* sur la leucémie murine P388. Cependant, des résultats récents montrent qu'il n'y aurait pas nécessairement parallélisme entre l'activité antipaludique et l'activité antitumorale, et qu'il est possible de trouver des quassinoïdes ayant un rapport dose toxique/dose efficace plus favorable.

Il nous a donc paru intéressant de vérifier l'activité antipaludique de quassinoïdes possédant d'autres types de squelette carboné, généralement moins toxiques. Nous avons entrepris l'étude des propriétés antiparasitaires des quassinoïdes de *Simaba cedron,* une espèce utilisée dans toute l'Amérique tropicale comme antipaludique, et dont ont été isolées par POLONSKY en 1962 la Cedronoline et la Cédronine, possédant un squelette carboné à 19 atomes de carbones.

Ces deux quassinoïdes ont été isolés à nouveau d'un échantillon récolté en Guyane française, et leur identification confirmée par leurs caractéristiques physiques et spectrales (RMN 2D, masse, UV).

Nous présentons ici l'activité antipaludique *in vitro* et *in vivo* de la Cédronine, le quassinoïde le plus actif des deux, comparée à sa toxicité sur cellules Kb et sa toxicité aigüe sur souris.

Remerciements:
Nous remercions le laboratoire de Pharmacognosie de la Faculté de Pharmacie de Reims, France (Dr G. MASSIOT et C. LAVAUD) pour la réalisation des spectres RMN 2D

Cytotoxic dysorone E and other constituents of the leaves of *Dysoxylum roseum* (Meliaceae) from New Caledonia

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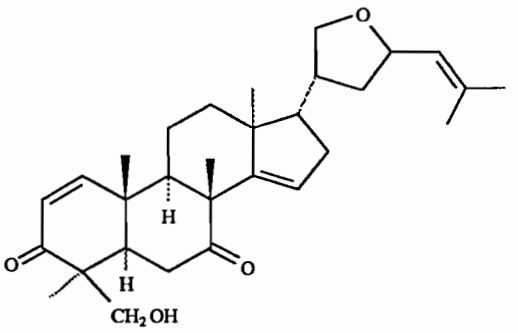
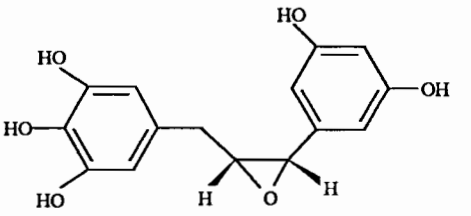
Chromatographic fractionation of the MeOH extract of the leaves of *Dysoxylum roseum* (Meliaceae) as directed by cytotoxic activity in standard KB cell test led to the isolation of five apotirucallane triterpenes and a 1,3-diarylpropan-2,3-epoxide. These novel compounds were characterised on the basis of their spectral data and named dysorone A-E and dysoroxide respectively. Only the major compound dysorone E showed significant cytotoxic activity with ED_{50} of 3.5 mg/ml.



Dysorone E



Dysoroxide

Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

New indole alkaloids from two Malaysian *Kopsia : K. larutensis* and *K. lapidilecta* (Apocynaceae)

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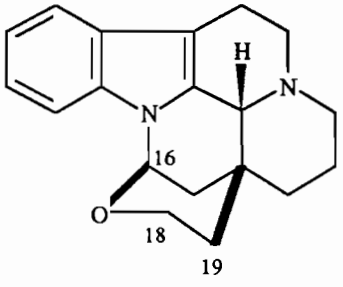
In the framework of the French-Malaysian cooperation program for the study of medicinal plants in Malaysia, two species have been collected.

Barks and stems of *Kopsia larutensis* King and Gamble (Batu Gajah forest collection) has been extracted to afford six alkaloids two of which are new : larutensine **1** and eburnaminol. The spectral data indicated that both have the eburnane skeleton and the former has an additional ether linkage between C-16 and C-18.

Kopsia lapidilecta King and Gamble (Mersing forest collection) gave two new alkaloids. It appears from the spectral data, that these alkaloids, under investigation, have a skeleton similar to aspidofractinin.



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Third Pacific-Asia Symposium on biologically active natural products, Noumea, New Caledonia, August 26-30th, 1991

Graciliflorin, an antibiotic indole alkaloid from a Malaysian Rubiaceae, *Pavetta graciliflora*

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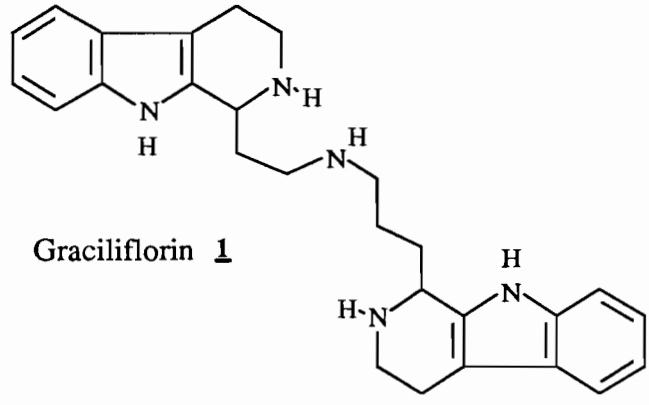
A crude alkaloid extract from *Pavetta graciliflora* exhibits a significant activity against G+ and G- bacteria. Bioassay-guided fractionation leads to the isolation of a polar substance, called graciliflorin.

Spectral data (MS, NMR) lead to the hypothesis of a bis-ß-carboline structure with a amino-alkyl chain linking the 2 parts.

Synthesis by Pictet-Spengler between tryptamine and the convenient bis aldehyde let us confirm the structure of graciliflorin as **1**.

1 is active *in vitro* on *S.aureus* but inactive *in vivo*



Graciliflorin **1**

Biological studies of the seeds of *Ipomoea muricata* (Jacq.) Linné (Convolvulaceae)

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Decoction of the seeds of *Ipomea muricata* (Jacq). Linné was traditionally used for different skin ailments such as ulcers, wounds, lesions, boils, burns, etc. It was also claimed to produce analgesic and anti-inflammatory effects.

The crude extract of the seeds was subjected to in vitro microbiological, pharmacological and toxicological tests (toxicity tests, analgesic, anti-inflammatory and anti-mutagenic investigations) to prove its efficacy against microorganisms and its safety.

The investigations indicated that it is an effective antimicrobial and analgesic agent and at certain doses possesses anti-mutagenic properties.

A pharmaceutical dosage form, an ointment, was prepared. An open clinical trial was performed on volonteer human subjects. The drug was found to be effective on skin ailments. It also produced analgesic effects and was found to have no side effects.

Synthesis and structure activity relationship of piperazine derivatives antagonists of platelet activating factor (PAF)

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Platelet-Activating factor (PAF) is a phospholipidic mediator involved mainly in accute inflammation and blood platelet aggregation phenomena.

Its biological effects are mediated through the activation of a high affinity binding site whose biochemical components remained unknown for a long time.

Detailed electronic studies on both synthetic and natural antagonists, choosen for their high activities and the difference between their chemical structures, led us to propose the hypothesis of a bipolarized receptor site made of a cylinder showing two positive areas and an hydrophobic pocket which may constitute three anchoring points for a given antagonist.

Construction of this model was based on the analysis of the 3-D electrostatic potential maps calculated for these products, which are synthetic neolignan, a triazolothienobenzodiazepine and two compounds extracted from chinese plants (Gingkolide and Kadsurenone).

Confirmation of this receptor structure can be logically achieved by the synthesis of molecules able to fit to this binding site. Preliminary studies have shown that N,N' bis (3,4,5) trimethoxybenzoyl piperazine inhibits blood platelet aggregation inducedby PAF (IC50=1,78µM).

Introduction of a hydrophobic chain of various lenfhts in position 2 of the piperazinic moiety leads to a sensible increase of the antagonist activity.

In the course of this talk, we will examine the 3-D potential maps which enabled the construction of the receptor hypothesis. Synthetic pathways of the N, N' bis (3,4,5) trimethoxybenzoyl piperazines substituted in position 2 will be shown as their biological results in blood platelet aggregation induced by PAF. A structure activity relationship study of these compounds will be discussed.

Perforatin : an unusual tetranor-triterpenoid from *Harrisonia perforata*

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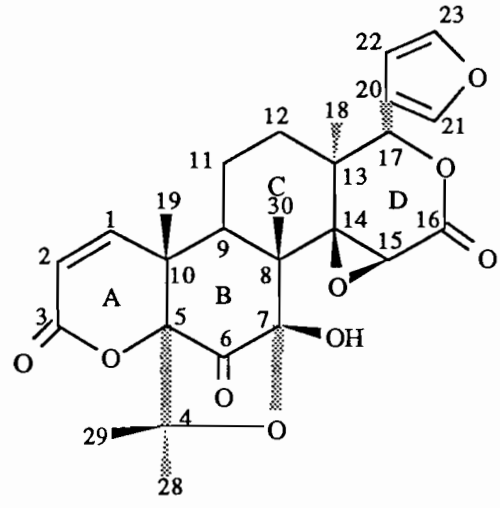
Extraction of the dried leaves of *Harrisonia perforata* (Blanco) Merr (Simaroubaceae), has yielded a novel tetranortriterpenoid (limonoid), perforatin, of the obacunol class. The unusual structure of the compounds was determined by a combination of spectroscopic analysis and the X-Ray method. Comments are made on the stereochemistry and the related limonoid Harrisonin.



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Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

Total Synthesis of 19-hydroxytubotaiwine. Assignment of absolute configuration to a natural isomer.

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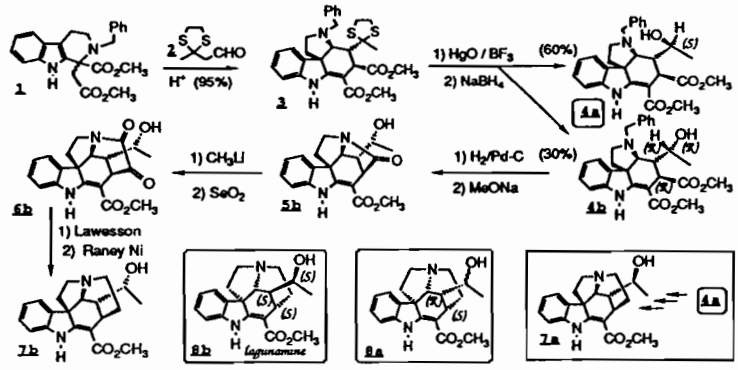
We present, herein, the total synthesis of two isomers of racemic 19-hydroxytubotaiwine from tryptamine [1]. Full NMR and X-ray studies of an intermediate allows us to describe the absolute configuration of one of the two natural isomers .

Our strategy follows a general route, devised to synthesize alkaloids of the *Strychnos* type[2], which already led to the total synthesis of tubotaiwine[3]. It starts from tetrahydro-ß-carboline diester **1**[4] reacting under acid catalyzed conditions, with aldehyde **2** to give **3**[5]. Complete analyses of **4b** (NMR and X-ray) and **4a** are the basis of the assignment of relative configurations to **7b** and **7a** as depicted below :

Two isomers of 19-hydroxytubotaiwine **8a** and **8b** have been isolated from *Alstonia angustiloba*[6] and, more recently a third one, named "**lagunamine**", from *Alstonia scholaris*[7]. Thus, this synthesis proves the absolute configuration of these alkaloids as follows : **8b** and **lagunamine** are identical to each other and superimposable to **7b** (in all their properties but specific rotation), and **8a** must have, at least, a 20-*R* configuration and its C-19 configuration cannot be deduced from this work.



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3. Total syntheses of (±) and (-)-tubotaïwines, Legseir,B., Henin, J., Massiot, G. and Vercauteren, J., submitted to publication.
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5. All compounds are prepared in their racemic form, but for easier reading in the schemes, we have depicted only one enantiomeric series (the relative configuration for all carbons in this series is the one depicted on the formulae).
6. Both isomers have been isolated for the first time, from *Alstonia angustiloba*, by L. LeMen-Olivier : a)Zèches, M., Ravao, T., Richard, B., Massiot, G., LeMen-Olivier, L., Guilhem, J. et Pascard, C., *Tetrahedron Lett.*, **1984**, *25*, 659. b)Ravao, T., Alcaloïdes de deux *Alstonia* indonésiens *: A. angustiloba* et *A. pneumatophora* (Apocynacées) , University of Reims 1985.
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Third Pacific-Asia Symposium on biologically active natural products, Noumea, New Caledonia, August 26-30th, 1991

Isolation and Structural Elucidation of the Major Component from *Pleiocarpidia* sp. : " 3-epi-19-S-dihydrocadambine "

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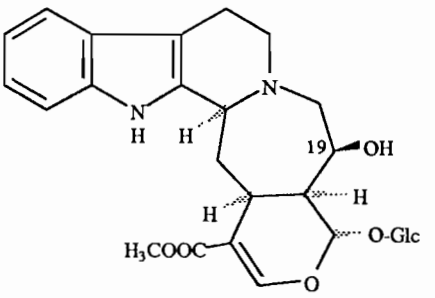
Heteronuclear reverse ^{1}H-^{13}C correlation spectroscopy and ROESY NMR experiments along with comparison with literature data for dihydrocadambine derivatives, allow us to propose a 3α-H isomer structure to the heterosidic indole alkaloid isolated from the fruits of *Pleiocarpidia novo sp.* (Rubiaceae) collected in Malaysia.

This *Pleiocarpidia* is a small tree owning orange berries with 5 multi-seeded carpels. The crude ethanolic extract of these fruits (100 mg; 0.6 g/kg) is separated by multiple preparative TLC ($CHCl_3$-CH_3OH 15% and $CHCl_3$-Acetone-CH_3OH, 45/45/20 v/v). The major component **1** (30% of totum) is a pale yellow amorphous compound (mp = 186-8°C) at R_F = 0.55 ($CHCl_3$-CH_3OH; 65-35 on silica gel with a greenish-yellow spot on spraying with Ce IV^+ sulfate.



1 = 3-α-dihydrocadambine

A complete set of physical and spectroscopic data is then collected from **1** : $[a]_D$ = -98° (c = 0.2; CH_3OH); UV ΝΑΛΑLΛVS0(MeOH;max nm) (log e) = 223 (4.54); 280 (3.80); IR n cm^{-1} = 3347, 1693, 1637; MS (FAB $^+$) = 547 (M+H $^{+\cdot}$) and mostly NMR that are presented : COSY ^{1}H-^{1}H allows signal proton assignment to the genin moiety. Reverse heteronuclear direct (^{1}J) and long range (^{2} and ^{3}J) experiments (HMQC, HMBC) fully confirm these attributions along with those for all ^{13}C ones. Comparison with "gathered data" from the literature[2] shows very much similarity between our compound **1** and 3-α-dihydrocadambine depicted above. ROESY correlation spectroscopy confirm the absolute configuration of C-19 as (*S*) for which coupling constants are not obvious arguments for that purpose.

Such a compound has a strong hypotensive effect on injection into rats[3].

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Troisième Symposium sur les substances naturelles d'intérêt biologique de la région Pacifique-Asie, Nouméa, Nouvelle Calédonie, 26-30 Août 1991

Strategies in the search for novel bioactive secondary metabolites

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It is argued that living organisms remain a prime source for the procurement of novel organic structures that can be used as lead compounds in the search for new pharmaceutical and agrochemical products. A major consideration is how best to obtain such novel compounds.

In this paper, I examine the strategies adopted within the Strathclyde Institute for Drug Research. These include the use of ethnobotanical information, bioassay-guided screening, unstructured phytochemical investigation and studies based on ecological observations. Attention will be drawn to the value of having a well organised and established biological resource base.

It is concluded that no one approach can be recommended to the exclusion of others. Other circumstances will often dictate which approach is adopted.

Third Pacific-Asia Symposium on biologically active natural products, Noumea, New Caledonia, August 26-30th, 1991

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