

Biochemical Research in the Philippines: 1990-An Overview¹

TOXINS WHICH ALTER THE EXPRESSION OF GENETIC INFORMATION CYL Sylianco and WT Shier

Genotoxins, and inhibitors of RNA and protein synthesis are discussed.
Chapter 5 of Handbook of Toxinology. Marcell-Dekker Inc. pp 337-421 (1990).

GOD'S DESIGN OF BIOMOLECULES CYL Sylianco

Structural design of bases of nucleic acids, coenzymes and energy-rich systems are discussed in relation to their functions in the living cell.

In Nature, Science and Values. Readings 11. Edited by Fr. Rector N. Castillo. University of Sto. Tomas. Tomas Press. pp 25-32 (1990).

MUTAGENIC AND ANTIMUTAGENIC ACTIVITIES OF PHILIPPINE MEDICAL PLANTS CYL Sylianco

Research on mutagenic and antimutagenic activities of Philippine medical plants were discussed. Proceedings the Symposium on Development of Drugs from Plants. UNESCO 101-112 (1990).

INHIBITORY EFFECTS ON NON-INDUCED MUTATION CYL Sylianco

Data on inhibitory effects on spontaneous mutation by vitamins and mineral ions are presented.

Acta Manilana 38:57-63 (1990)

DIETARY EFFECTS ON GENOTOXINS AND ANTIGENOTOXINS CYL Sylianco

Protein deficient diets enhanced genotoxic activity of benzo(a)pyrene and dimethylnitrosamine. Coconut oil diets inhibited the genotoxicity of some carcinogens.
Acta Manilana 38: 65-74 (1990).

INHIBITORY EFFECTS OF SOME MEDICAL PLANTS ON GERM CELL GENOTOXICITY CYL Sylianco, R Abian and FR Blanco

Germ cell genotoxicity of methylmethanesulfonate, tetracycline, and chloromycetin were inhibited by decoctions from bayabas, kogon, kamias, makabuhay, allungay, yana, sambong, tanglad and ulasimang bato.

Science Diliman 3: 1-7 (1990).

¹Abstracts of papers published by PBS members in 1990. This compilation is by no means complete and for this issue, there is no clearcut classification of the abstracts except, perhaps, by laboratory where research was conducted. Interest readers can contact the authors directly or send their inquiries to the Editor of the PBS Bulletin.

GENETIC TOXICOLOGY
CY LIM-Sylianco

1990. 240 pages. Published by NAST (National Academy of Science and Technology of the Philippines).

MODERN BIOCHEMISTRY
CY LIM-Sylianco

4th edition. 1990. 707 pages. Published by Aurum Technical Books. QC

DIVERSITY OF CONUS NEUROPEPTIDES

Baldomero M. Olivera, Jean Rivier, Craig Clark, Cecilia A Ramilo,
Gloria P. Corpuz, Fe C. Abogadie, E. Edward Mena, Scott R. Woodward,
David R. Hillyard, Lourdes J. Cruz

Conus venoms contain a remarkable diversity of pharmacologically active small peptides. Their targets are ion channels and receptors in the neuromuscular system. The venom of *Conus geographus* contains high-affinity peptides that act on voltage-sensitive calcium channels, sodium channels, *N*-methyl-D-aspartate (NMDA) receptors, acetylcholine receptors, and vasopressin receptors; many more peptides with still uncharacterized receptor targets are present in this venom. It now seems that the *Conus* species (approximately 500 in number) will each use a distinctive assortment of peptides and that the pharmacological diversity in *Conus* venoms may be ultimately comparable to that of plant alkaloids or secondary metabolites of microorganisms. The cone snails may generate this diverse spectrum of venom peptides by a "fold-lock-cut" synthetic pathway. These peptides are specific enough to discriminate effectively between closely related receptor subtypes and can be used for structure-function correlations.

Science 249: 257-263 (1990)

CONANTOKIN-G: A NOVEL PEPTIDE ANTAGONIST TO THE
***N*-METHYL-D-ASPARTIC ACID (NMDA) RECEPTOR**

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Conantokin-G is a 17 amino acid peptide isolated from the venom of the fish-eating snail *Conus geographus* which produces hyperactivity when injected into the brains of adult mice. We show that this peptide is a selective *N*-methyl-D-aspartate (NMDA) antagonist based on its ability to block NMDA-induced elevation of cGMP in rat cerebellar slices *in vitro* (IC₅₀ = nM), but not kainic acid-induced elevations. This inhibition could not be overcome by increasing the NMDA concentration, indicating non-competitive inhibition. Conantokin-G displayed no affinity for binding sites for thienylcyclohexypiperidine, various glutamate subclasses or those for several other neurotransmitters/neuromodulators. This peptide, however, enhanced [³H] glycine binding to rat forebrain membranes but not to spinal cord membranes. The activity profile of the peptide in various assays indicates that it is a novel type of non-competitive NMDA antagonist.

Neuroscience Letters 118:241-244 (1990)

CONSTANT AND HYPERVARIABLE REGIONS IN CONOTOXIN PROPEPTIDES

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Conotoxins are small cysteine rich peptides found in the venom of the predatory cone snails (*Conus*) which have proven to be useful high affinity ligands for various receptors and ion channels. The first cloning data for conotoxins, reported here, were obtained for the King-Kong peptide, a 27 amino acid conotoxin found in the venom of the cloth-of-gold cone, *Conus textile*. Analysis of cDNA clones of the King-Kong peptide revealed a family of related toxin transcripts. Three different propeptide cDNA sequences were obtained; only one of these encoded sequence for the King-Kong peptide. The other cDNA sequences encoded two different peptides (KK-1 and KK-2). When the predicted propeptide sequences are compared, well defined conserved and hypervariable regions can be identified. The hypervariable regions comprise four regions between Cys residues in the final peptide toxins; the remainder of the propeptide sequences, i.e. the excised N-terminal regions and the disulfide bonded Cys residues, are highly conserved. We suggest that the conserved regions may direct the formation of a specific disulfide configuration in the King-Kong family of conotoxins.

The EMBO Journal 9, 1015-1020

TOXINS AND OTHER BIOLOGICALLY ACTIVE PEPTIDES OF *CONUS GEOGRAPHUS* VENOM

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Proc. 2nd Eur Asia Conf. Chem., 1990

CONOTOXINS: TARGETED PEPTIDE LIGANDS FROM SNAIL VENOMS

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Several hundred species of *Conus* snails produce a wide array of small (12-30 AA) and mostly tightly disulfide bonded peptide ligands with high affinity for a diverse set of receptor and ion channel types. These include nicotinic acetylcholine receptors, neuronal calcium channels, muscle sodium channels, vasopressin receptors, and *N*-methyl-D-aspartate (NMDA) receptors. Some general features of the structure, function, and evolution of biologically active peptides isolated from *Conus* venom are presented.

In: Marine Toxins: Origin, Structure, and Molecular Pharmacology. S. Hall and G. Strichartz, ACS Symposium Series, American Chemical Society, West DC pp 256-278.

CONANTOKIN-T

A γ -CARBOXYGLUTAMATE CONTAINING PEPTIDE WITH N-METHYL-D-ASPARTATE ANTAGONIST ACTIVITY

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Conantokin-T, a 21-amino acid peptide which induces sleep-like symptoms in young mice was purified from the venom of the fish-hunting cone snail, *Conus tulipa*. The amino acid sequence of the peptide was determined and verified by chemical synthesis. The peptide has 4 residues of the modified amino acid, γ -carboxyglutamate (Gla). The sequence of the peptide is: Gly-Glu-Gla-Gla-Tyr-Gln-Lys-Met-Leu-Gla-Asn-Leu-Arg-Gla-Ala-Glu-Val-Lys-Lys-Asn-Ala-NH₂. Conantokin-T inhibits N-methyl-D aspartate (NMDA) receptor-mediated calcium influx in central nervous system neurons. This observation suggests that like conantokin-G (a homologous *Conus* peptide with recently identified NMDA antagonist activity) conantokin-T has NMDA antagonist activity. A sequence comparison of conantokins-T and -G identifies the 4 Gla residues and the N-terminal dipeptide sequence as potential key elements for the biological activity of this peptide.

J Biol Chem 265:6025-6029 (1990)

PEPTIDES FROM CONUS VENOMS WHICH AFFECT CA⁺⁺ ENTRY INTO NEURONS

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The venoms of fish-hunting *Conus* contain paralytic conotoxins and an unprecedented variety of other biologically-active peptides. Particularly noteworthy are peptides which inhibit calcium entry into neurons, The *conantokins* and *w-conotoxins* which target NMDA receptors and voltage-sensitive Ca channels respectively.

The conantokins contain at least four residues of γ -carboxyglutamate (Gla), a post-translationally modified amino acid. Conantokins are folded in an α -helical conformation and bind acidic membranes in a calcium-dependent manner. Upon envenomation, conantokins may concentrate on appropriate membranes and target to peripheral fish NMDA receptors located on such membranes.

The *w-conotoxins* are disulfide bonded peptides; a comparison of several *w-conotoxins* sequences reveals considerable sequence variability. Recent studies with *w-conotoxins* have also revealed a developmental specificity in mice. The results suggest that elements controlling breathing in neonatal mammals, but not in adults, are *w-conotoxin* sensitive.

J. Toxicol-Toxin Reviews 9:179-202 (1990)

REDUCTION OF HEAVY METAL CONCENTRATIONS IN LIQUIDS BY *RHIZOBIUM* POLYSACCHARIDES

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Five strains of *Rhizobium* sp isolated from the roots of *Leucaena leucocephala* (ipil-ipil) and one from *Glycine max* (soybean) were observed to synthesize prodigious amount of extracellular mucilaginous polysaccharides. These strains were tested to find out how effective they were in reducing the concentrations of heavy toxic metals, Hg, Cd and Pb from liquid media. The use of rhizobial polysaccharides as sequestering agents for heavy metals will provide a new technology for environmental management.

The rhizobial strains were grown in yeast extract mannitol broth (YEMB) containing the heavy metal at two concentrations, 500 ug and 1,000 ug/L. These concentrations are 10 to 100 times higher than the WHO limits for these toxic metals. Concentrations of 10, 20, 30 and 100 ppm of the metals were also studied. The period of incubation ranged from 4 to 10 days, after which the cell culture was centrifuged and the supernatant analyzed for its heavy metal content by isotachophoretic methods of analysis.

Results show a drastic reduction of heavy metal concentration in the supernatant to below WHO limits of 10 ug/L and 100 ug/L Pb. Transmission electron micrographs of *Rhizobium* spp grown in YEMB with heavy metals showed dense extracellular areas.

ASEAN J. Technol. Develop. 6(2) (1989) 75-86

ADSORPTION OF MERCURY BY *RHIZOBIUM LOTI* STRAIN BL1 80

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Rhizobium loti strain BL 1 80, an isolate from the roots of *Leucaena leucocephala* was observed to produce prodigious amounts of mucilaginous polysaccharides. This strain was cultured in varying concentrations of Hg^{2+} and found to survive even at high concentrations of 100 ppm Hg (10^{-4} M).

After the cells of a 7-day old culture was removed, the concentration of Hg^{2+} in the supernatant was reduced to about 50% from the original concentration of 15 ppm Hg. The amount of Hg^{2+} reduced in the supernatant depends on the cell density, cell age, and time of agitation.

Optimum growth parameters for mass culture of BL1 80 show a maximum cell count after 3-4 days incubation period and maximum polysaccharide production after the 7th to 10th day of incubation. Mass cell growth is enhanced by increasing inoculum level from 2% to 4%.

Transmission electron micrographs of LB1 80 cells grown in YEMB alone showed a thick outer cell layer. YEMB with Hg^{2+} showed the whole cell surface covered with a dense layer.

The Philippine Journal of Biotechnology Vol. 1, No. 2 149-159 (1990)

**DT - DIAPHORASE POLYMORPHISM IN A NATURAL POPULATION
OF *RATTUS RATTUS MINDANENSIS***

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DT-diaphorase polymorphism was detected in a sample of 128 *Rattus rattus mindanensis* collected at random from farmers in the towns of Bay, Lumban, Pakil, Pangil, and Mabitac in Laguna, Philippines.

Crude liver extract and hemolysates of rats treated subcutaneously with 0.0005% warfarin were used. Both liver and erythrocyte DT-diaphorase activity were highly variable indicating a wide phenotypic variability. The average level of liver DT-diaphorase activity observed is near the mean value of heterozygous resistant *Rattus norvegicus*.

DT-diaphorase isozyme polymorphism was found in liver and erythrocyte using disc-gel electrophoresis. Fifteen DT-diaphorase isozymes were resolved with crude extract from liver and 10 isozymes from hemolysates. Similarly indices based on the electrophoretic zones identified genetic similarity only within the B group with respect to erythrocyte DT-diaphorase. Heterogeneity was detected among all groups with values higher in liver DT-diaphorase than in erythrocytes. Liver DT-diaphorase isozymes in groups C, D and E were significantly different from A and that of B from C.

Grouping was based on cluster analysis of data on prothrombin in intraperitoneally injected rats, a parameter for resistance associated with DT-diaphorase.

The Philippine Agriculturist Vol. 73, No. 3 & 4, 419-433 (1990)

**FREQUENCY OF ABO BLOOD GROUPS AND ITS CORRELATION
TO SOME GENETIC DISORDERS AMONG FILIPINOS IN DIFFERENT
BARANGAYS OF LOS BAÑOS, LAGUNA**

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Blood typing using anti-A and anti-B blood grouping sera among 3,771 Filipinos in 12 Barangays of Los Baños, Laguna showed a frequency of 22.81% "A", 28.98% "B", 6.36% "AB" and 41.85% "O" blood types. Based on a X^2 test, the calculated gene frequencies of 0.16 A, 0.19 B, 0.65 O and genotype frequencies of 0.02 AA, 0.04 OO BB, 0.42 OO, 0.06 AB, 0.21 AO and 0.25 BO were found to follow Hardy-Weinberg equilibrium.

Significant correlations were found between Diabetes mellitus and blood group "A" ($C=0.057$), epilepsy and blood group "A" ($C=0.052$) and keloid tendency with group "A" and "AB" ($C=0.06$).

Philippine Journal Science VOL. 119 NO. 4, 315-322 (1990)

**ISOHEMOGLOBINS OF THE MILKFISH, *CHANOS CHANOS* (FORSKALL)
(*OSTEICHTHYES: CHANIDAE*) COLLECTED FROM TWO SITES IN LAGUNA DE BAY**

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Blood samples from 56 ten-month-old milkfish *Chanos chanos* (forskall) were analyzed using polyacrylamide disc gel electrophoresis (PAGE). Twenty-eight fish were collected from fishpens in each site: Calamba, Laguna (Site A) and Alabang, Metro Manila (Site B).

A total of 23 isohemoglobins were observed from all the samples examined. Multiple isohemoglobins in complex and heterogeneous patterns ranging from 5-13 in Site A and 5-14 in Site B were manifested. Isohemoglobin 1, the fastest moving band which is uniquely expressed in Site B, has an average Rf of 0.984 while isohemoglobin 23, the slowest moving band found in both sites, had an average Rf of 0.138. Cathodal isohemoglobins were predominantly expressed in both sites while anodal isohemoglobins occurred in higher frequency in Site B than in Site A.

Statistical analysis for equality of variance of the relative mobility of isohemoglobins revealed that there is a significant difference in the variability of isohemoglobins between the two sites at 1.0 % level of significance. The difference in variability observed cannot, however, be correlated to the difference in oxygen content of both sites since water analysis showed that both contain roughly 7.30 mg/L of dissolved oxygen. The possible difference could be attributed to other factors in the environment, such as pH. Water in Alabang, with a lower pH than the water in Calamba, seems to favor the high incidence of anodal isohemoglobins in the former.

U.P. Los Baños Journal Vol. 1, No. 1, 69-83 (1990)

**PHYSICOCHEMICAL BASIS FOR HARDSEEDEDNESS IN MUNG BEAN
(*VIGNA RADIATA* (L.) WILCZEK)**

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Hard seeds ranged from 0 to 3.8% in four varieties and four seed lots of mung bean from commercial sources. Uncooked normal and hard seeds were indistinguishable although, after boiling for 30 min, the hard seeds remained uncooked and were hard, brownish, and wrinkled. The hardness and seed coat thickness of hard seeds were twice those of normal seeds. Hard and normal seeds had similar chemical proximate composition except for fiber content, which was 9-25% greater in the hard seeds. The seed coats of hard seeds had 12% higher fiber content, 7 times more lignin, and 23% higher silica than the normal. The amino acid composition and pectic substances content of the two types of mung bean were similar. Histochemical analysis and scanning electron microscopy revealed a more rigid and highly structured palisade layer in the hard seed than in the normal seeds.

J. Agric. Food Chem. 38:29-32 (1990)

PROXIMATE CHEMICAL COMPOSITION OF SEVERAL
PHILIPPINE INDIGENOUS LEGUMES

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Protein (18 to 30%) and carbohydrates (50 to 60%) are the major constituents of the mature seeds of 33 samples comprising seven legume species. The protein content of *Canavalia ensiformis*, (L.) DC, *Canavalia gladiata* (Jacq.) DC, *Mucuna pruriens* (L.) DC or *M. cochinchinensis* and *Clitoria ternata* L. range from 28 to 30% while *Vigna umbellata* (L.) DC had the lowest (17.42 to 17.56%). Fat content range from 1.2 to 3.7% in all 33 samples. *Dolichos lablab* L., *Phaseolus lunatus* (L.) Macf., and *Canavalia gladiata* (Jacq.) DC had had the largest (5-6%) difference in protein and carbohydrate contents among accessions.

The proximate composition of the immature and mature leaves and pods were also obtained: moisture (70-90%), carbohydrates (15-18%), proteins (3-10%), fibers (2%), fat (2-4%) and ash (0.5-4%).

The Philippine Agriculturist 73, 69-74 (1990)

OLIGOSACCHARIDES IN SEVERAL PHILIPPINE INDIGENOUS FOOD LEGUMES:
DETERMINATION, LOCALIZATION AND REMOVAL

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The oligosaccharides profile of raw mature seeds of seven different legumes indigenous to the Philippines was measured in 70% ethanol extracts of the seeds by thin layer chromatography using HPTLC plate and quantified by a densitometer. Based on the results, the legumes could be ranked according to decreasing oligosaccharides content or flatulence potential as follows: Sam-samping (*Clitoria ternatea*) > hyacinth bean (*Dolichos lablab*) > sabawel (*Mucuna pruriens*) > lima bean (*Phaseolus lunatus*) > swordbean (*Canavalia gladiata*) > rice bean (*Vigna umbellata*) > jack bean (*Canavalia ensiformis*). Sam-samping had 4.79% total oligosaccharides and hyacinth bean or batao, 3.66%. A jack bean accession had 1.79% oligosaccharides.

Simple processing methods were tested to detoxify the oligosaccharides. Soaking the batao seeds had no effect while boiling even resulted in a net 23-31% increase in the levels of raffinose, stachyose and verbascose. On the other hand, two min of dry roasting resulted in complete removal of oligosaccharides whereas germination resulted in about 30-40% decrease after 1 and 2 days, respectively.

Plant Food for Human Nutrition 40: 83-93, 1990

THE RELATION OF POLYPHENOLS AND POLYPHENOL OXIDASE TO THE FOOD QUALITY OF BANANA BUD

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Polyphenols in banana buds were found to consist of flavanan tannin (condensed tannin) as the major component; catechin, its oligomers, dopamine and dopa as minor components. Total phenol and vanillin-positive phenol were higher in the buds of cultivars Bungulan, Lacatan and Latundan and lower in the buds of cv. Saba. Polyphenol oxidase (PPO) in cv. Saba was localized in the male flower; 3 times less in the peduncle and bract: and about 30 and 300 times less in the peel and pulp of the fruit respectively. PPO activity in the male flower, peduncle and bract was inhibited by 0.13 M NaCL about 20 to 30% while that in the peel and pulp was inhibited about 40 to 50%. Buds of the other cultivars had similar levels of PPO which were inhibited about 20% by 0.13M NaCL. The Ki value for NaCL of PPO in the peel was estimated to be 0.28 M. The astringency, bitterness and color of banana buds were monitored before and after heating, NaCL treatment and squeezing. These are discussed with reference to the polyphenols and PPO focussing on food quality.

Nippon Shokuhin Kogyo Gakkaishi 37, 730-736 (1990)