天然物化学における単離について

ー海洋天然物化学 特に核酸 テトラトキソン及びテトラトキソン類化合物の分離についてー

韓国科学院 金 容海

Isolation, Purification and Structural study of Marine Natural Products of Nucleic Acids, Tetrodotoxin and Chiriquitoxin

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Summary Separation and structural study of biologically active marine natural products are discussed A rare nucleic acid doridosine was isolated from boneless sea animals, dorid nudibranch, Anisodoris nobilis digestive glands shown to cause prolonged reduced arterial pressure and heart rate in mamals The structure of doridosine was determined as N^1-methyl isoguanosine Potent neurotoxins of tetrodotoxin(TTX) and chiriquitoxin(CHTX) were isolated from Atelopus chiriquiensis frogs CHTX shows similar biological activity and has 392 molecular weight (73 higher than TTX (319)) Biologically active (anti histamine release effect) material was isolated from ligia exotica together with various nucleic acids and amino acids

1 Introduction

A new rare nucleic acid, N-methylnucleoside named doridosine identified as 1-methylisoguanosine was isolated from the shell-less marine dorid nudibranch Anisodoris nobilis, from the Australian sponge, Tadanis digitata [2,3] recently from the carribean carol Madaracis mirabilis [4] While, isoguanosine was isolated from dorid nudibranch Diaulula Sandigenses [5] Recently, inosine and unknown nucleic acid were isolated and conformed from Ligia exotica

Based on the hypothesis that shell-less marine mollusks, specifically dorid nudibranchs, might contain a toxic substance that protects them from predators, various tissue extracts of specimens were prepared [6] Aqueous extracts of these were subjected to a preliminary purification by dialysis followed by general toxicity by injection into mice and crabs Toxic extracts were studied in more detail in several standard pharmacological preparations From these studies it emerged that extracts of the digestive glands of anisodoris nobilis had the unusual property,

among extracts from marine animals, of producing hypotension and bradycardia in mammals within a few seconds of intravenous injection in anesthetized rats These extracts caused a marked and prolonged reduction in heart rate and a sharp drop in systolic blood pressure The activity of the extracts could be followed conveniently by testing on the isolated, spontaneously beating, guinea pig atria We reported that the cardioactive component of the digestive gland of Anisodoris nobilis is a new N-mehtylpurine riboside that we named doridosine Doridosine was assigned the structure of 1-methylisoguanosine (1) based on its spectroscopic properties The fuction and origin of this compound in these marine organisms are unknown It is known that dorid undibrnchs feed principally on sponges,[7 8] but our preliminary studies have failed to detect any doridosine in several food sources of Anisodoris nobilis

Doridosine causes reduced arterial pressure and reduced heart rate in mammals in a manner that is qualitatively similar to adenosine but with an unusually long duration of action [1 9 10] It also shows skeletal muscle relaxant and hypothermic activity [9 10]

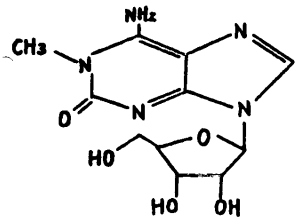
It is interesting that various nucleic acids of inosine, uracil, guanosine and 2-deoxyguanosine, and anti-alergy compound were isolated from ligia exotica So small sea animal contains large amounts of nucleic acids and various amino acids Low pressure liquid column chromatography (Bio-Gel P2 and Sephadex G10) and JAI (Japan Analytical Institute) HPLC (column GAIGS 320 20 x 500 mm) combination was very effective for the separation of various amino acids and nucleic acids

Chiriquitoxin (CHTX, 1) was first isolated in 1975 from the Costa Rican frog Atelopus chiriquiensis [11] On the basis of ^{1}H NMR[12] and mass spectra[13], its structure was postulated to differ from that of tetrodotoxin (TTX, 2) only with

respect to the substituent at C-6 2 is a potent neurotoxin of puffers[14] and newts,[15] and is an important neurobiological tool [16] Among derivatives and natural analogs of 2,[17] 1 is unique in being as potent as 2 in lethality to mice[11] and in blocking the voltage-gated sodium channel,[18] whereas all others have markedly reduced biological activities [19] Earlier work with 1 was hampered by a scarcity of material and by difficulties in separating it from co-existing 2 In late June 1988, renewed collection of the frogs was successful Using the paradigm which led to structural determination of natural analogs of 2 in newts[17a] and in puffers [17b c d] We report here the structure of 1

2 Doridosine (1-methyl isoguanosine)

This marine natural product was isolated from dorid nudibranch, Anisodoris nobilis (digestive glands) As a rare nucleic acids of isoguanosine derivatives, its structure was elucidated as 1-methylisoguanosine Separation and purification were done as show below



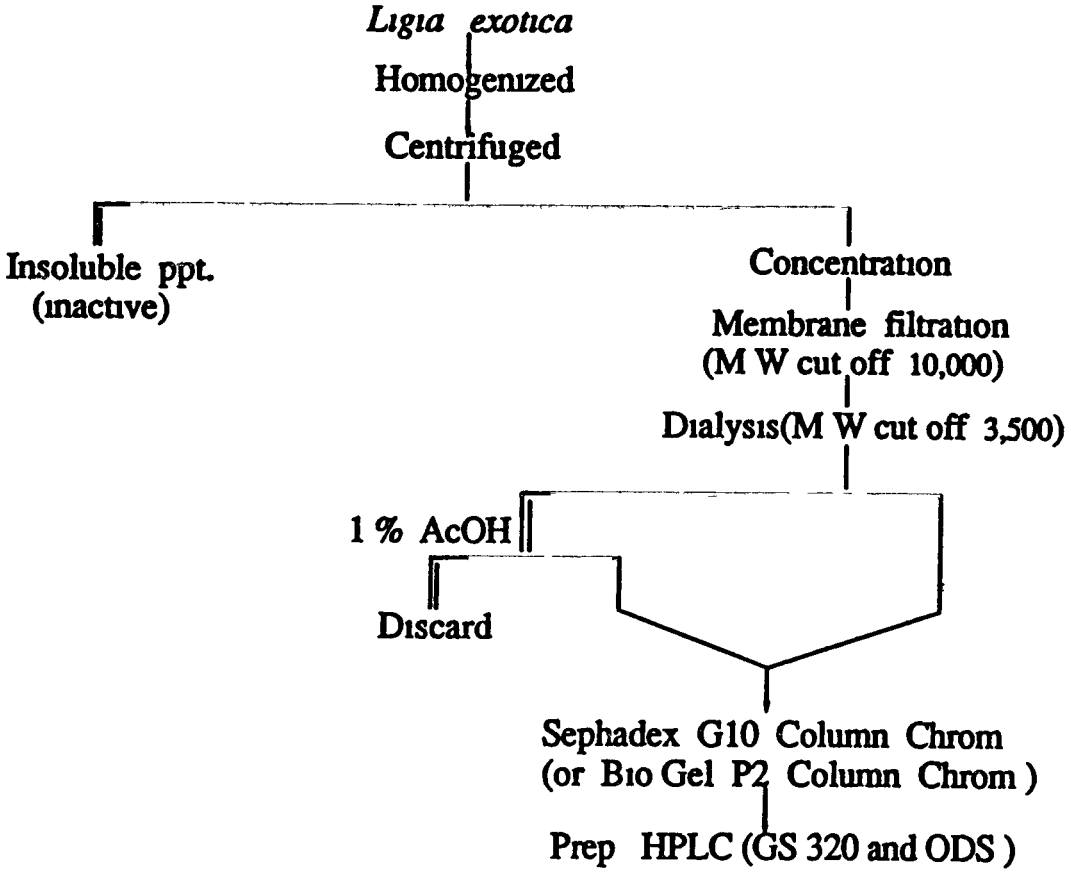
Doridosine

** Isolation and Pusification of Doridosine

Digestive glands (Anisodoris nobilis)
(128_g)
← Homogenized
← dialized (MW Cut off 6 000-8 000)
← Concentrated lyophilized
← MeoH added centifuged
discard
Bio-Gel P-2 Chromatographed
Silica-gel chromatographed
Crystallized with t-BuoH MeoH : H_2O
(2 1 1)
Recrystallized with t-BuoH MeoH
(21 mg)

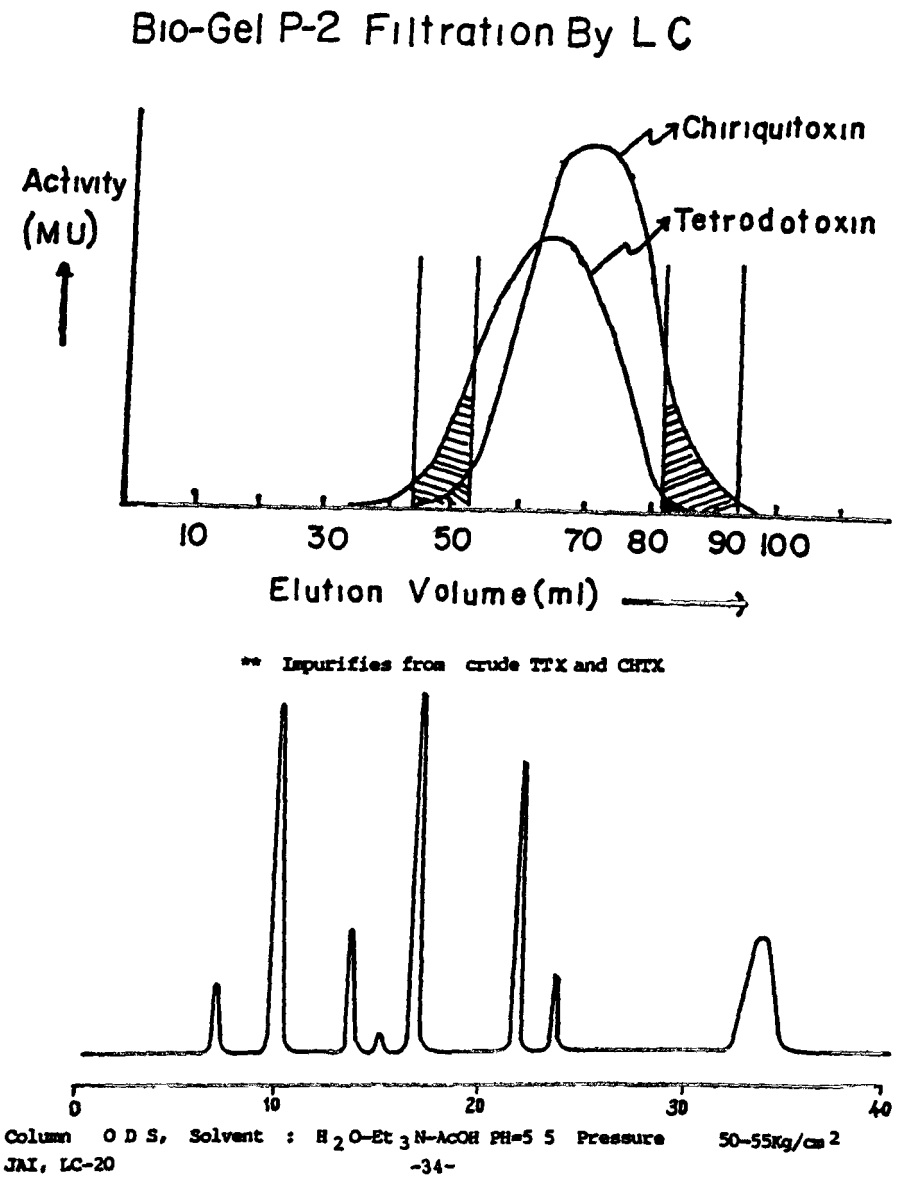
3 Separation and purification from Ligia exotica

Biologically active compounds (inhibitory effects on histamine release), amino acids, and nucleic acids were separated and identified

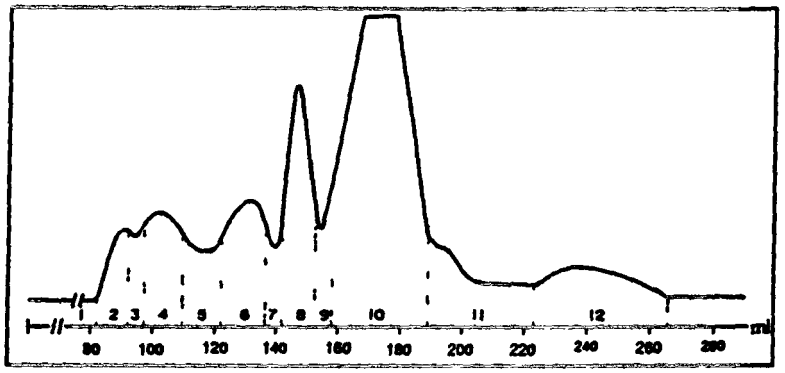


4 Separation and characterization of Tetrotoxin (TTX) and Chiriquitoxin (CHTX)

summary The structure of chiriquitoxin, a tetrodotoxin analog isolated from the Costa Rican frog Atelopus chiriquiensis, was elucidated on the basis of NMR data In the structure 11-CH_2OH of tetrodotoxin was replaced by a $CH(OH)CH(NH_2)COOH$ group



Sephadex G 10 Column Chromatography



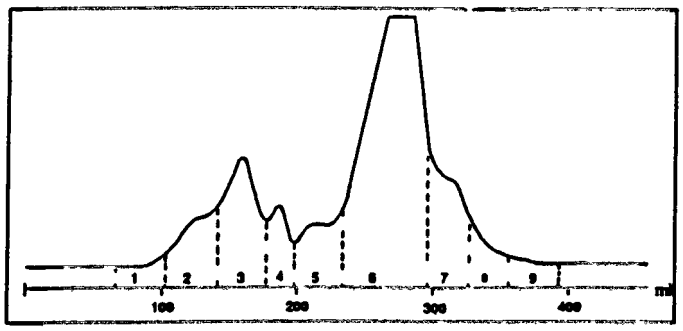
Column	Sephadex G 10 (1 9 X 110 cm)
Detector	UV 254
Eluent	H_2O
Flow rate	20 ml/hr
Sample	2g/3ml H_2O

PREPARATIVE HPLC SEPARATION

Column	JAI GS 320 (20X500 mm)
Detector	UV 254 and RI
Eluent	MeOH H_2O = 70 30
Flow rate	3 ml / mn

Sample No	Isolated Compounds
6	Betaine, Ala ,Val ,Pro Ile , Leu, Gly, Unknown 1, 2
8	Phe , Uridine, Unknown 3 , 4
10	Inosine, Unknown 5, 6
12	Trp-1, Guanosine, Unknown 7 2 -Deoxyguanosine
ppt	Tyrosine

LOW PRESSURE LC



Column	Bio Gel P2 (20X300 mm)
Detector	UV 254
Eluent	Et_3N-AcOH-H_2O pH 6 2
Flow rate	3 ml/min

Fraction NO	1	2	3	4	5	6	7	8	9
Amounts (mg)	-	16	49	59	7	19	7	4	9

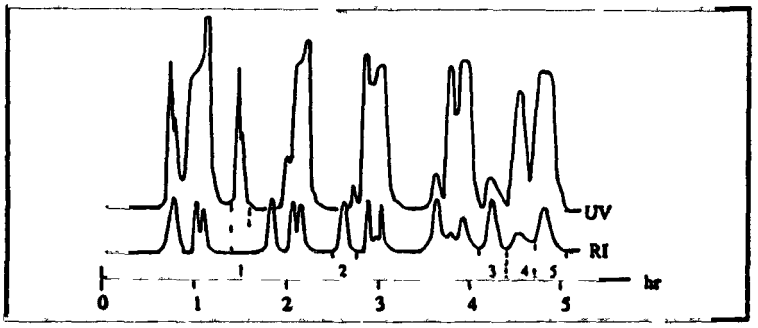
BIOLOGICAL ACTIVITY

Inhibitory effects of *Ligia Exotica* fractions on Histamine release from Rat Peritoneal Mast Cells induced by compound 48/80

Treatment	Concentration	Histamine release (%)	Inhibition (%)
Control		68 3	
Blank		3.6	
1		26.0	6.4
2	0.5	19 4	75.6
3	0.5	12.6	86.1
4	0.5	12.5	86.2
5		42.9	39.3
6	0.5	12.4	86.4
7	0.5	18.1	77 6
8	0.5	60.3	12.3
9	0.5	32.5	55.2

*mg/ml Concentration of compound 48/80 ($5 X 10^{-7}$ g/ml)

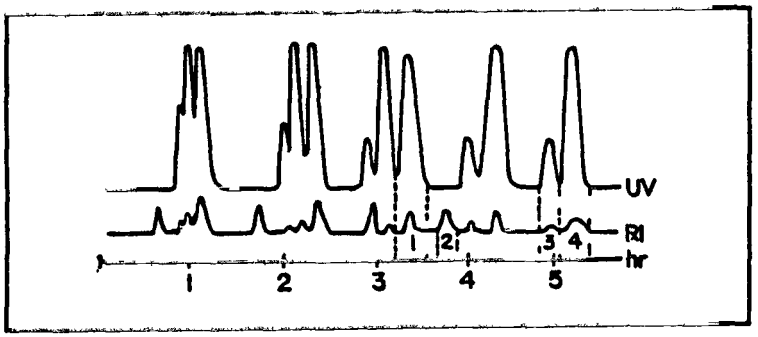
RECYCLE PREPARATIVE HPLC



Sample Sephadex G 10 Column Fraction 10

Fraction No	Isolated compounds
1	Unknown 7
2	Ile , Leu
3	Phenylalanine
4	Unknown 8
5	Inosine

RECYCLE PREPARATIVE HPLC



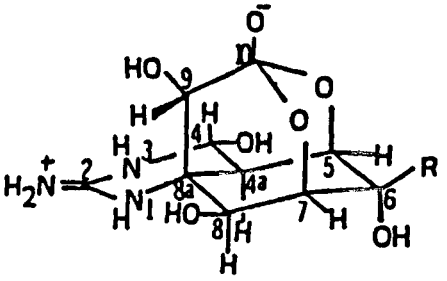
Sample Sephadex G 10 Column Fraction 12

Fraction No	Isolated compounds
1	Trp-1
2	Unknown 7
3	2-Deoxyguanosine
4	Guanosine

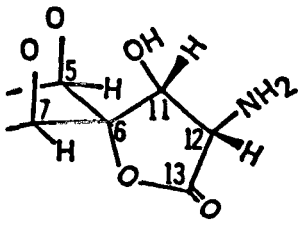
NMR spectral data of CHTX and CHTX-13,6-lactone

	CHTX (1)*		CHTX-13,6-lactone (4)**	
	C	H	C	H
2	156 6		156 7	
4	75 2	5 51 (d 9 4)	75 3	5 53 (d 8 5)
4a	40 5	2 31 (d 9 4)	41 7	2 16 (d 8 5)
5	73 5	4 40 (br s)	70 6	4 71 (br s)
6	72 1		84 3	
7	81 1	4 39 (br t)	77 7	4 30 (br t)
8	72 7	4 40 (br s)	72 8	4 19 (d 1 5)
8a	59 3		59 5	
9	70 9	4 00 (s)	70 7	4 03 (s)
10	111 0		111 1	
11	70 3	4 90 (d 1 8)	69 7	5 20 (d 5 5)
12	58 1	4 27 (d 1 8)	-	4 85 (d 5 5)
13	174 1		173 0	

^{13}C NMR *75 5 MHz (GN-300), **100 MHz (JEOL GSX-400) $^{13}CD_3COOD$ as 22 4 ppm ^{1}H NMR *300 MHz (GN-300), **400 MHz (JEOL GSX-400), CHD_2COOD as 2 06ppm
Solvent * 4%CD_3COOD/D_2O, ** 1%TFA, 4%CD_3COOD/D_2O (45°C)
- Unassignable carbon due to an exchange of H-12 with D (^{13}C NMR spectrum of 4 was measured after keeping 1 in 1%TFA, 4%CD_3COOD/D_2O for one month at 5°C)



R



4 CHTX-13,6-lactone

	R
1 CHTX	11 12 13 $CH(OH)CH(NH_2)COOH$ *R* *S*
2 TTX	11 CH_2OH
3 11-norTTX-6,6-diol	OH

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