カキ,アサリ,ホタテガイからの麻ひ性貝毒の抽出法の検討

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Extraction of Paralytic Shellfish Poisons from Whole Oyster, Whole Short-Necked Clam, and Scallop Digestive Gland

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Repeated acidic methanol extractions of paralytic shellfish poison-containing oysters and short-necked clams followed by a hot HCl extraction resulted in obtaining 2-3 times the toxicity realized by the standard mouse bioassay procedure. This effect was not observed with repeated acidic ethanol extractions nor with scallop digestive glands. Secondary methanol extractions of residues from the evaporation of the primary alcoholic extracts of shellfish generally gave poor recoveries of activity and fraction with lower specific activities, but extraction of the residues from the hot HCl extracts generally gave good recoveries and an enhancement of specific activity.

The ever increasing need to utilize the seas as a source of human nutrition and the increasing occurrence of paralytic shellfish poisons (PSP) throughout the world have given impetus to the study of these very toxic natural substances. The standard mouse bioassay, in which an extract of the poisons is made by boiling a homogenized shellfish sample with an equal weight of 0.1 N hydrochloric acid for 5 min, is used throughout the world for the estimation of PSP levels. As part of a study to develop simple methods for the detection of PSP and to develop alternative methods of assay, the efficacy of several different procedures for the extraction of PSP from shellfish was studied.

Materials and Methods

Shellfish Samples

Frozen shucked oysters, *Crassostrea gigas*, were from Senzaki, Yamaguchi Prefecture; frozen shucked short-necked clams asari, *Tapes philippinarum*, were from Owase Bay, Mie Prefecture; and frozen scallop, *Patinopecten yessoensis*, digestive gland was from Ofunato Bay, Iwate Prefecture.

Mouse Bioassay

White mice weighing 17-21 g were injected intraperitoneally with 1 m*l* of extract or a dilution of an extract and death times noted. Mouse Units (MU) were calculated using the standard table of Sommer.[1] When extracts were below pH 3, they were brought up to pH 3-4 with 6 N NaOH before testing.

Extraction Procedures

The primary extraction procedures used in extracting the shellfish samples are described in Table 1. The secondary procedure used in extracting the residues resulting from the evaporation to dryness *in vacuo* of the primary extracts is described in the footnote of Table 3.

Table 1. Primary extraction procedures for extracting PSP from shellfish

Method	Details
H(0.1)S	Standard extraction procedure for the mouse bioassay. 50 m*l* 0.1 N HCl added to 50g of homogenized shellfish meat, and the mixture boiled for 5 min and centrifuged at 3000 rpm for 30 min.
H(0.1)	100 m*l* 0.1 N HCl added to 50 g of shellfish meat, the mixture homogenized for 2-5 min, heated at 75-80°C for 5 min, cooled rapidly, and centrifuged at 3000 rpm for 30 min.
H(0.05)	Similar to H(0.1) but using 0.05 N HCl.
U(100% M)	100 m*l* 100% methanol, containing 0.4m*l* conc. HCl per 100 m*l*, added to 50 g of shellfish meat, the mixture homogenized for 2-5 min, then centrifuged at 3000 rpm for 15-30 min.
U(80% E)	Similar to U(100% M) but using 80% (v/v) ethanol acidified to pH 2 with conc. HCl.

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Results

Comparison of Hot HCl Extraction Procedures

In Table 2, the results of primary extraction are shown. As seen in the table, extraction with twice (v/w) the amount of hot 0.1 N HCl at 75–80°C resulted in the extraction of 87% of the MU obtained in the standard bioassay procedure (lines 20, 21), which employs an equal amount (v/w) of 0.1 N HCl and boiling temperatures. When HCl extractions at 75–80°C were made on shellfish residues remaining after initial acidified methanol or ethanol extractions, 0.05 N rather than 0.1 N HCl was used in order to reduce the amount of NaOH needed to bring the pH up for bioassay. When extraction by acidified ethanol was followed by extraction with hot 0.05 N HCl at 75–80°C, a total of about 80% of the MU obtained by the standard assay procedure was extracted (lines 22–23, 28–29, 30–31).

Acidified Methanol Extraction

Repeated acidified methanol extractions of both oysters and short-necked clams followed by a hot 0.05 N HCl extraction resulted in a total number of MU which was 2–3 times the MU realized by the standard assay procedure. Using oyster meat the maximum activity was found in the 2nd or 3rd methanol extracts, after which, the activity gradually decreased (lines 9–13). In the

Table 2. Primary extraction of shellfish

Line No.	Material	Extraction procedure[*1]	Lot No.	MU in extract per g of shellfish[*2]	MU per mg of solids in extract	% recovery based on standard assay
1	Oyster	H(0.1)S	2	26		100
2	(whole meat)	U(100%M)	2	14	0.29	
3		U(100%M)		24	2.4	227
4		H(0.05)		21	0.16	
5		U(100%M)	2	18	0.33	
6		U(100%M)		14	1.09	327
7		U(100%M)		29	6.0	
8		H(0.05)		24	0.34	
9		U(100%M)	2	12	0.23	
10		U(100%M)		11	1.08	
11		U(100%M)		22	3.5	235
12		U(100%M)		8	3.7	
13		U(100%M)		3	1.8	
14		H(0.05)		5		
15		H(0.1)S	4	44		100
16		U(100%M)	4	22	0.39	
17		U(100%M)		23	1.4	191
18		U(100%M)		24	3.9	
19		H(0.05)		15	0.34	
20		H(0.1)S	3	39		100
21		H(0.1)	3	34		87
22		U(80%E)	3	19	0.25	82
23		H(0.05)		13	0.16	
24		U(100%M)	3	16	0.21	
25		U(100%M)		10	0.49	164
26		U(100%M)		18	2.36	
27		H(0.05)		20	0.06	
28		U(80%E)	1	14	0.26	82[*3]
29		H(0.05)		6	0.06	
30		U(80%E)	1	11	0.21	78[*4]
31		H(0.05)		8	0.06	
32		U(80%E)	1	6	0.12	
33		U(80%E)		1.3		63[*4]
34		U(80%E)		1.0		
35		H(0.05)		7	0.12	

Line No.	Material	Extraction procedure*1	Lot No.	MU in extract per g of shellfish*2	MU per mg of solids in extract	% recovery based on standard assay
36	Short-necked	H(0.1)S	2	4.6		100
37	clam	U(100%M)	2	3.0	0.06	
38	(whole meat)	U(100%M)		1.4	0.11	311
39		U(100%M)		3.8	0.71	
40		H(0.05)		6.1	0.28	
41		U(100%M)	2	4.2	0.07	
42		U(100%M)		1.4	0.12	343
43		U(100%M)		5.4	0.79	
44		H(0.05)		4.8	0.53	
45		H(0.1)	1	9.7		87*5
46		U(80%E)	1	2.2	0.06	51*6
47		H(0.05)		3.5	0.15	
48		U(80%E)	1	3.2	0.06	51*6
49		H(0.05)		2.5	0.10	
50	Scallop (digestive	H(0.1)S	2	438		100
51	gland)	U(100%M)	2	293	4.4	
52		U(100%M)		92	4.5	110
53		U(100%M)		52	5.9	
54		H(0.05)		43	0.98	
55		H(0.1)S	1	214		100
56		U(100%M)	1	96	1.38	62
57		H(0.05)		36	0.98	
58		U(80%E)	1	64	0.84	49
59		H(0.05)		41	1.06	

*1 Indented entries in this column indicate that the insoluble shellfish fraction resulting from the extraction of the immediately preceding line was re-extracted as specified.
*2 Residues from evaporation of extracts (from 50 g of shellfish) to dryness *in vacuo* were taken up in 50 m*l* of water and centrifuged at 3000 rpm (12,000 rpm, if not readily centrifuged) for 30 min to remove insoluble material and the supernates re-evaporated to dryness *in vacuo*.
*3 Based on results of lines 22, 23.
*3 Based on results of lines 28, 29.
*5 Based on results of lines 20, 21, comparing the H(0.1) to the H(0.1)S method in the oyster.
*6 Based on results of line 45.

case of the scallop digestive gland, however, successive methanol extractions followed by a hot 0.05 N HCl extraction (lines 51–54) resulted in approximately the same number of total MU as obtained by the standard assay procedure.

In the case of the oyster or short-necked clam, the specific activity of the 2nd and 3rd methanol extracts was considerably higher than that of the 1st methanol extract or the hot 0.05 N HCl extract (*e. g.*, lines 5–7, 9–12). Such a marked trend was not observed with the scallop digestive gland.

Acidified 80% *Ethanol Extraction*

Repeated acidified 80% ethanol extractions of oyster meat followed by a hot 0.05 N HCl extraction resulted in a recovery of about 60% of the activity found by the standard assay procedure (lines 32–35), and, in general, a single ethanol extraction followed by a hot 0.05 N HCl extraction gave 50–80% of the mouse bioassay value with the oysters, short-necked clams, and scallop digestive gland.

Secondary Extraction with Methanol

The results of the extraction with methanol of the residues resulting from the evaporation *in vacuo* of the primary extracts of the shellfish are shown in Table 3. Extraction of the residues resulting from the initial methanol or ethanol extracts gave a recovery of 38–64% of the activity with a considerable decrease in the specific activity. This was true for all three shellfish sources. On the other hand, methanol extraction of the residues from the hot HCl extracts gave recoveries of 42–93%, with occasional recoveries greater than 100%, and generally enhanced specific activities.

Table 3. Secondary extraction of residues from the primary extraction of shellfish

Material	Primary extraction			Secondary extraction*1			
	Procedure*2	MU per 50 g shellfish	MU per mg solids in extract	MU per 50 g shellfish	MU per mg solids in extract	% extraction of primary residue	Fold increase in specific activity
Oyster (whole meat)	U(100%M)	700	0.29	451	0.26	64	−0.1
	U(100%M)	—	—	—	—	—	—
	H(0.05)	1050	0.16	781	1.54	74	9.6
	U(80%E)	678	0.26	273	0.15	40	−0.4
	H(0.05)	308	0.06	545	1.62	177	27
	U(80%E)	547	0.21	171	0.10	31	−0.5
	H(0.05)	378	0.06	206	0.21	54	3.5
Short-necked clam (whole meat)	U(100%M)	150	0.06	77	0.04	51	−0.3
	U(100%M)	—	—	—	—	—	—
	U(100%M)	—	—	—	—	—	—
	H(0.05)	305	0.28	128	0.61	42	2.2
	U(80%E)	277	0.055	106	0.03	38	−0.5
	H(0.05)	215	0.095	159	0.12	74	1.3
	U(80%E)	110	0.06	42	0.03	38	−0.5
	H(0.05)	175	0.15	136	0.30	78	2.0
Scallop (digestive gland)	U(100%M)	4800	1.38	1924	0.73	40	−0.5
	H(0.05)	1800	0.98	1392	1.72	77	1.8
	U(80%E)	3175	0.84	1407	0.61	44	−0.3
	H(0.05)	2065	1.06	1924	2.05	93	1.9

*1 The secondary extraction was carried out by extracting the residue resulting from the evaporation *in vacuo* of the primary extract from 50 g of shellfish material with 50 m*l* of methanol and filtering. The methanol insoluble fraction was washed with 25 m*l* of methanol and the washing combined with the methanol extract. If the insoluble material was bulky, the methanol wash was repeated.

*2 Indented entries in this column indicate that the insoluble shellfish fraction resulting from the extraction of the immediately preceding line was re-extracted as specified.

Discussion

Methanol is commonly used as a solvent for extracting water-soluble small molecular weight substances from natural sources, affecting a separation of these substances from water-soluble large biomolecules, such as proteins and polysaccharides. When oysters and short-necked clams were extracted repeatedly with acidified methanol and finally with hot 0.05 N HCl, it was found that generally 2–3 times the MU as determined by the standard mouse bioassay procedure directly on the shellfish could be realized. Most of this activity was found in the methanol extracts. When a similar procedure was tried on oysters with acidified 80% ethanol, a solvent system commonly used for the extraction of PSP from various natural sources, well under 100% of the MU as determined by the standard assay procedure was realized. It was found that secondary methanol extractions of residues remaining from the evaporation to dryness of primary hot 0.05 N HCl extracts of the shellfish (line 5, Table 3) and the tertiary extraction with methanol of residues from these secondary methanol extracts (148%, 153%, unpublished data on short-necked clams) occasionally gave recoveries of activity well over 100%. When tertiary extractions with 96% ethanol were carried out on residues remaining from similar secondary methanol extracts, recoveries of activity were always well under 100% (59% for the oyster; 36% for the scallop digestive gland; unpublished data). The marked difference in behavior between methanol and 80 or 96% ethanol in extracting PSP activity might suggest factors other than solubility in these solvents as being responsible for the increased activity found in the case of the methanol extractions. Seemingly greater than 100% recoveries of PSP activity have been observed in the past,[2–4] and are generally difficult to explain, even when taking into account the inaccuracies of the mouse bioassay. In this study they occurred in every instance in the case of the repeated acidified methanol extraction of oysters and short-necked

clams, and this suggests that a yet unrecognized factor is operating to produce this result. Methanol may somehow be potentiating the activity of PSP so as to produce more MU than are actually present in nature in the toxic shellfish. The results of this study may be useful in determining the forms in which PSP occurs.

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References

1) B. W. Halstead: Poisonous and Venomous Marine Animals of the World, Vol. 1, U. S. Gov't Printing Office, 1965, p. 201.
2) E. J. Schantz, J. D. Mold, D. W. Stanger, J. Shavel, F. J. Riel, J. B. Bowden, J. M. Lynch, R. S. Wyler, B. Riegel, and H. Sommer: *J. Amer. Chem. Soc.*, **79**, 5230–5235 (1957).
3) A.A. Casselman, R. Greenhalgh, H.H. Brownell, and R. A. B. Bannard: *Can. J. Chem.*, **38**, 1277–1290 (1969).
4) L. J. Buckley, M. Ikawa, and J. J. Sasner, Jr.: in "Proceedings of the First International Conference on Toxic Dinoflagellate Blooms, Nov. 4–6, 1974, Boston, Mass." (ed. by V. R. LoCicero), Mass. Sci. and Tech. Foundation, Wakefield, Mass., 1975, pp. 423–431.