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Purification of domoic acid from toxic blue mussels (*Mytilus edulis*) and phytoplankton

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ABSTRACT

Domoic acid was the primary neurotoxin in blue mussel (*Mytilus edulis*) that caused poisoning in humans. Further research showed that the algae, *Nitzschia pungens*, was the source of this toxin. In this study, a method for the extraction and purification of domoic acid from contaminated mussels and phytoplankton was developed. Domoic acid was extracted from these sources by treatment with a mixture of chloroform and methanol (1:2, v/v). The resulting extract was subjected to ultrafiltration through a PMI Millipore filter, followed by repeated high-performance liquid chromatography on a reversed-phase column. The purity and yield of domoic acid prepared by this method are compared with two previously described methods of extraction. The current method is relatively simple, rapid, and results in improved recovery with comparable purity of domoic acid.

INTRODUCTION

Domoic acid was identified as the primary toxin in mussels that caused poisoning in humans in 1987 [1]. Those intoxicated by the shellfish toxin initially displayed gastrointestinal symptoms (nausea, abdominal cramps, diarrhea), followed by neurological disorders, *i.e.* headache, confusion and loss of memory [2,3]. The contaminated mussels were harvested from the east coast of Prince Edward Island, Canada. In a previous communication we reported data indicating a close relationship between *in vivo* toxicity measured by the mouse bioassay and domoic acid concentration in toxic blue mussels. Both *in vivo* toxicity and domoic acid were concentrated in the hepatopancreas of contaminated mussels, and declined in parallel over time under either natural habitat conditions in Cardigan River and under laboratory conditions where the contaminated mussels in storage tanks were flushed with fresh water [4,5]. Also, *in vivo* toxicity and domoic acid in contaminated mussels were direclty related to chlorophyll concentration in the hepatopancreas [4,5]. These results are consistent with the observations of other investigators [1,6–8] and support the conclusions that (1) domoic acid was the primary toxin that caused human poisoning in 1987 and (2) that the algae

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Nitzschia pungens was the primary source of domoic acid in contaminated mussels.

This occurrence of human poisoning prompted the development of methods for rapid isolation and measurement of domoic acid from contaminated mussels. Initially, extraction of the mussel toxin was attempted by procedures that were used for the extraction of paralytic shellfish poisoning toxin. This involved homogenization of tissue in 0.1 *M* HCl and boiling for 5 min, followed by analysis by high-performance liquid chromatography (HPLC) [9–11]. Later it was found that domoic acid was unstable in acidic conditions, and extraction of domoic acid in water was recommended to improve the stability and yield of domoic acid [10,11].

The water extraction of domoic acid was considered reliable, but required extensive clean-up procedures prior to HPLC [10]. An effective extraction method was sought to improve the yield of domoic acid during purification. In the present study, we compared three extraction procedures in terms of yield and purity of domoic acid. This resulted in the development of a simple and rapid method for the extraction and purification of domoic acid from contaminated mussels and from phytoplankton.

EXPERIMENTAL

Materials

Materials were purchased as indicated: PM1 ultrafiltration membranes (1000 dalton exclusion limit) and stirred cells from Amicon Division of W. R. Grace, (Danvers, MA, USA); chloroform, methanol, acetonitrile, isopropanol and trifluoroacetate, all HPLC grade, from BDH (Dartmouth, Canada); precoated cellulose plates, acetone, acetic acid, ethyl acetate and phosphoric acid from Fisher Scientific (Dartmouth, Canada); collidine, ninhydrin, dihydrokainic acid, amino acid standard kit, domoic acid and Coomassie blue from Sigma (St. Louis, MO, USA); *n*-butanol from Johns Scientific (Toronto, Canada); 9-fluorenylmethylchloroformate (FMOC) from Aldrich (Milwaukee, WI, USA); boric acid from Anachemia (Montreal, Canada); domoic acid from Diagnostic Chemicals (Charlottetown, Canada). Deionized water was obtained by filtering distilled water through a Milli-Q system (Millipore, Bedford, MA, USA). Toxic mussels were kindly provided by the Department of Fisheries and Oceans (Charlottetown, Canada).

The HPLC system contained Gilson pumps (Model 302), a Gilson ultraviolet detector (Model 116), Gilson datamaster (Model 620) connected to 704 HPLC system manager software on an Apple II-C computer. A Shimadzu fluorometer (Model RF 535) was used for detection of fluorescent amino acid derivatives.

CD_1 female mice were purchased from Charles River Labs. (Montreal, Canada) or bred at the Atlantic Veterinary College (Charlottetown, Canada).

Extraction methods for domoic acid

Water extraction. The tissue was homogenized in an equal volume of water, and the homogenate was boiled for 10 min. The resulting mixture was allowed to cool at room temperature for 5 min and centrifuged for 10 min at 1500 *g* in a table top centrifuge (Beckman, Model TJ-6); the supernatant was decanted. The residue was re-extracted twice by the addition of one half volume of water (w/v), vortex-mixed vigorously and recentrifuged as before [10].

Acid extraction. The tissue was homogenized in an equal volume of 0.18 *M* HCl and, after the pH of the homogenate was adjusted to 3.5 with HCl, boiled for 7 min, allowed to cool to room temperature and centrifuged for 10 min at 1500 *g*. To study the efficiency of extraction method to remove domoic acid, the tissue residue after the first treatment was extracted twice more in one half volume of water (w/v).

Extraction with organic solvent. The tissue was homogenized in an equal volume of deionized water using a Ten Broeck glass homogenizer for small volumes and a Brinkman Polytron homogenizer for larger volumes. This homogenate was mixed with three volumes of chloroform–methanol (1:2, v/v) and vortex-mixed for 2 min. One volume of chloroform was added followed by vortex-mixing for 1 min; one volume of water was added and the solution vortex-mixed again for 1 min. The resulting mixture was centrifuged for 10 min at 1500 *g*. The top aqueous phase containing domoic acid and other water-soluble components was removed and methanol was driven off by blowing nitrogen through the mixture [5,12].

Analysis of domoic acid by high-performance liquid chromatography

The pre-purified extract (20 μl) was injected onto a C_{18} reversed-phase column (Pecosphere, 5-μm C_{18}, 15 cm × 0.46 cm I.D.) fitted with a Microbondapak C_{18} cartridge guard column (Waters, 5 μm C_{18}, 5 mm × 5 mm I.D.). The mobile phase consisted of acetonitrile–distilled, deionized water (12.5:87.5, v/v), pH adjusted to 3.0 with phosphoric acid, and previously filtered through 0.2-μm organic filter paper and degassed for 15 min by flushing with nitrogen gas. The mobile phase was pumped at a flow-rate of 0.75 ml/min and the eluent was monitored for absorption at 242 nm by a UV detector. Domoic acid eluted between 8 and 10 min post-injection and was completely resolved from other components (Fig. 1). Peak areas were integrated and calibrated with a primary standard of domoic acid purchased from the National Research Council's Laboratory, (Halifax, Canada). Analysis of domoic acid was accurate within 5% according to the collaborative study organized by Health and Welfare Canada.

Estimation of proteins

Proteins were measured by the method of Bradford [13] using bovine serum albumin (Fraction V) as a standard.

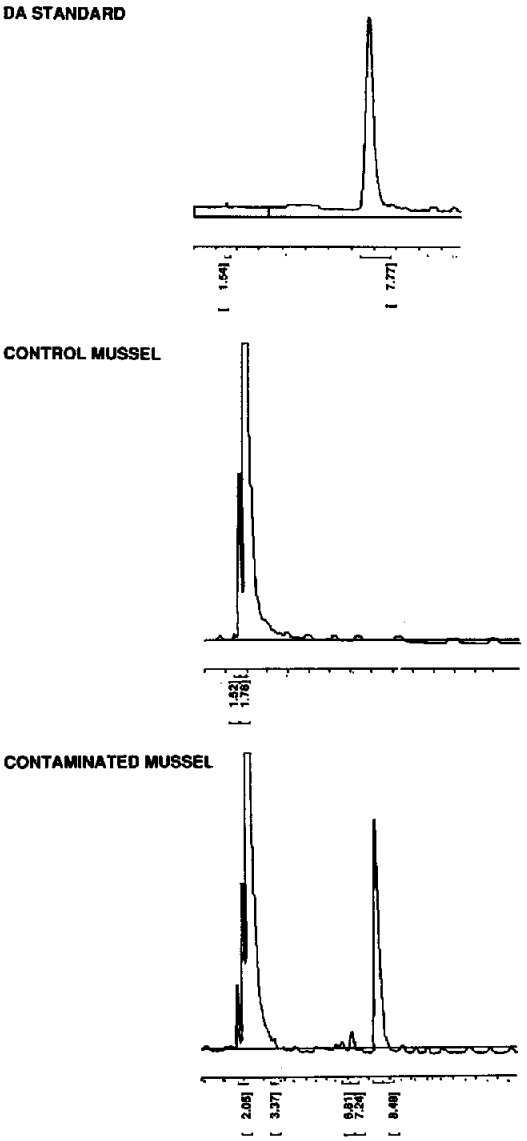

Fig. 1. HPLC profile of a primary standard of domoic acid (top panel), the extracts of control (middle panel) and toxic blue mussel (bottom panel) prepared with chloroform and methanol as described in Experimental.

Purification of domoic acid

Contaminated blue mussels. Digestive glands from toxic mussels were dissected and stored frozen at −80°C until processed. The digestive glands were homogenized and extracted in a solvent containing chloroform–methanol (1:2, v/v) as described above [5]. The phases were separated and the upper phase containing

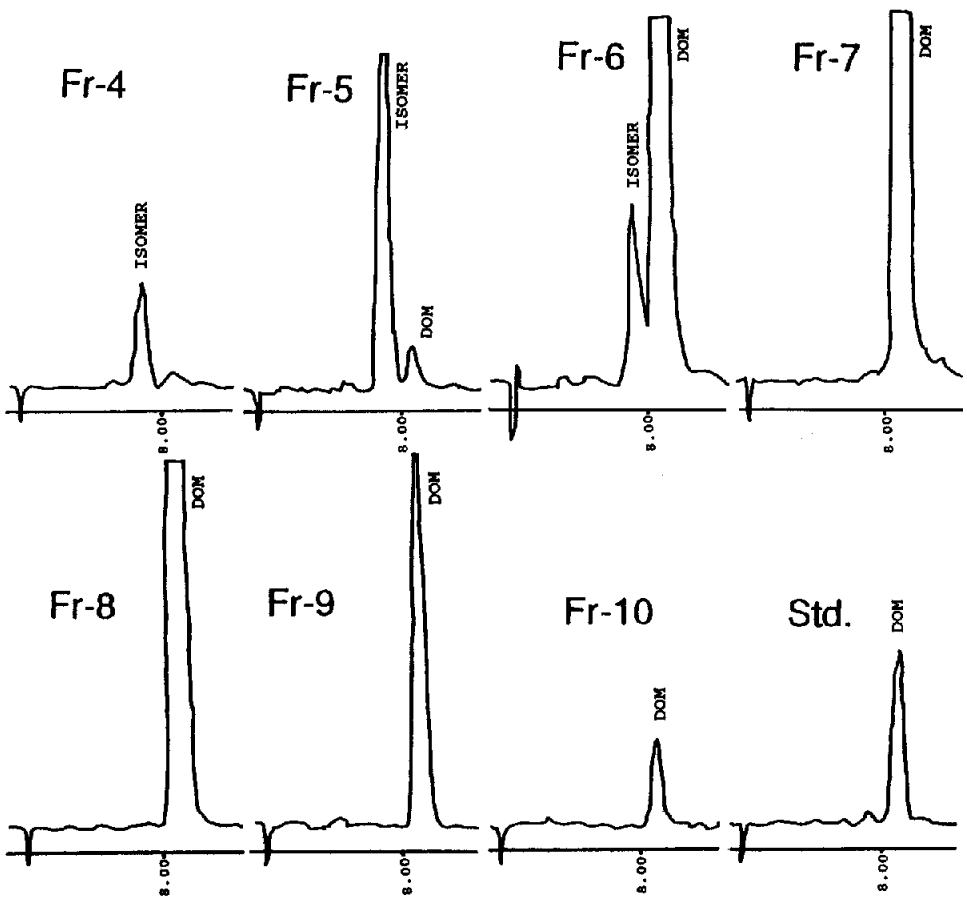

Fig. 2. HPLC analysis of pooled fractions from preparative HPLC. All chromatographic conditions are described in Experimental. DOM = domoic acid.

domoic acid was removed. A stream of nitrogen gas was blown into the pooled aqueous phase to drive off methanol. The remaining aqueous phase was frozen in liquid nitrogen and lyophilized overnight in a Labconco bench-type freeze dryer connected to a two-stage high vacuum. The residue was suspended in 2 ml of deionized water and the suspension subjected to ultrafiltration through PMl Millipore filter which retained molecules with molecular weight greater than 1000 dalton.

Ultrafiltration was performed in a plexiglass cell at 5°C by applying 200-kPa pressure with nitrogen gas. The solution in the cell was stirred automatically using a magnetic stirrer. Two rinses of 2 ml each of water were performed to recover any domoic acid adsorbed to the filter. The filtrate was lyophilized and reconstituted in 1 *M* HCl to yield 10 mg domoic acid per ml. The pH was monitored with pH paper to ensure that it was about 1.

This partially purified preparation of domoic acid was injected in 200-μl injections onto a reversed-phase column (Vydak 10-μm C_{18} reversed phase, 25 cm $\times$ 1.0 cm I.D., Technical Marketing Assoc. Mississauga, Canada) with a preguard column (Guard-Pak precolumn module with μBondapak C_{18} Guard-Pak precolumn inserts, Waters Division of Millipore, Mississauga, Canada) and subjected to HPLC with a mobile phase containing acetonitrile–distilled, deionized water (12.5:87.5, v/v), pumped at a flow-rate of 3.0 ml/min. Fractions of 1 min were collected. All operations were carried out at room temperature. Four to five runs were carried out, fractions pooled from different runs and an aliquot subjected to analytical HPLC to determine its composition (Fig. 2). Fractions containing domoic acid were pooled and lyophilized to obtain a powder-like domoic acid preparation. This preparation was examined for purity by analytical HPLC, UV–VIS absorption detection by a diode-array spectrophotometer (Hewlett-Packard Model 8452A) and by HPLC analysis of fluorescent derivatives of amino acid [6].

Phytoplankton (Nitzschia pungens). Domoic acid in toxic mussels appeared to originate from algae in estuaries, specifically *Nitzschia pungens* [4,7]. Since the phytoplankton cells are relatively fragile, extraction of domoic acid from the toxic phytoplankton was performed.

Phytoplankton containing domoic acid were collected from the Cardigan River using a net towed behind a motor boat. The phytoplankton obtained were identified morphologically as predominantly *Nitzschia pungens* species [14]. Samples were stored frozen at -30°C until utilized.

Phytoplankton samples (5 g each) were taken from a wet slurry in sea water. The slurry was mixed with a mixture containing chloroform–methanol (1:2, v/v) and centrifuged at 1500 *g* for 10 min to separate phases. The upper aqueous methanolic phase containing domoic acid was collected. Methanol from the pooled upper phase was evaporated off under a stream of nitrogen. Aqueous samples were lypophilized, and the residue was resuspended in a smaller volume of distilled, deionized water and subjected to ultrafiltration through PM1 Millipore filters. The filtrate was lyophilized and the residue resuspended in 1 *M* HCl to yield 10 mg domoic acid per ml. This solution was injected in 200-μl injections onto the HPLC column, and the components were eluted with the mobile phase consisting of acetonitrile–water (12.5:87.5, v/v) as described above. Fractions of 1 min were collected and analysed for domoic acid by analytical HPLC and UV–VIS absorption detection using a diode-array spectrophotometer.

Assessment of purity of domoic acid preparation

Analytical HPLC. A small volume (10 μl) of domoic acid preparation was subjected to analytical HPLC and the profile examined for domoic acid and other peaks. The presence of a major peak with a retention time corresponding to the primary standard of domoic acid was considered to be a reliable indicator of pure domoic acid (Fig. 1).

UV–VIS absorption by diode-array spectrophotometry. The domoic acid preparation was scanned from 190 to 830 nm by a diode-array spectrophotometer (Hewlett-Packard Model 8452A). As domoic acid absorbs maximally at 242 nm, a large peak at this wavelength and absence of other peaks were used as an indicator of purity.

Amino acid analysis by thin-layer chromatography (TLC). The domoic acid preparation was subjected to one-dimensional TLC on cellulose-coated (250 μm thick) glass plates using a solvent system containing *n*-butanol–acetone–acetic acid–water (35:35:10:20, v/v). After development, the TLC plate was sprayed with a mixture containing ninhydrin (0.2 g per 100 ml) and collidine (2.5 ml per 100 ml) in isopropanol [15]. The appearance of one uniform spot indicates the purity of the preparation.

Analysis of fluorescent amino acid derivatives by HPLC. The domoic acid preparation was incubated with FMOC, and derivatives of amino acids were subjected to HPLC following the method of Pocklington *et al.* [6]. This technique is more sensitive than the analytical HPLC technique described earlier, with a detection limit of 0.5 μg/g.

Measurement of toxicity by mouse bioassay

Mouse bioassays were performed to estimate toxicity in important HPLC fractions. Three mice weighing 20–24 g were injected intraperitoneally with 1 ml of each of test sample or the vehicle in controls and observed until death or for 4 h [9]. The times of onset of scratching behaviour and death were recorded. The purified domoic acid and standard (Diagnostic Chemicals) were dissolved in 0.85% saline, 10 m*M* citrate (pH 3.5), to maintain the osmolality and buffering capacity. Control mice received injections of the same vehicle (see Table III).

Statistical methods

Analysis of variance (ANOVA) and Student's *t*-tests were used to test for significant differences in the extraction methods. Statistical calculations were performed on Minitab software (version 6.1.1, Minitab, 1987).

RESULTS

The homogenization of digestive glands from contaminated mussels in a mixture of chloroform–methanol (1:2, v/v), followed by addition of distilled, deionized water, allowed separation of lipids in the chloroform phase, domoic acid plus other water-soluble components in the aqueous methanolic phase, and the denatured proteins at the interphase between the chloroform and aqueous methanolic phases. Treatment of mussels or phytoplankton with chloroform–methanol mixture extracted 75–80% of domoic acid from the tissue in one treatment, whereas similar treatment of mussel tissue with either water or 0.18 *M* HCl removed only 49 and 34%, respectively, of total domoic acid (Table I). Apparently, two extrac-

TABLE I

EXTRACTION OF DOMOIC ACID FROM THE DIGESTIVE GLANDS OF BLUE MUSSELS (*MYTILUS EDULIS*) BY DIFFERENT SOLVENTS

Results are expressed as mean ± S.D. for three independent extractions with different solvents. CM = chloroform–methanol; W = distilled water; A = 0.18 *M* HCl.

Extract	Domoate concentration (μg/ml)	Percentage of total	Proteins (μg/ml)	Specific activity domoic acid/protein (mg/mg)
Whole homogenate	1492	100		
CM-1	1145 ± 141	77	3259 ± 503	0.35
CM-2	180 ± 19	12	604 ± 53	0.29
CM-3	25 ± 1	2	229 ± 46	0.11
W-1	726 ± 49[a]	49	4611 ± 270[a]	0.16
W-2	338 ± 20	29	3960 ± 345	0.09
W-3	253 ± 8	16	2025 ± 317	0.12
A-1	513 ± 52[a]	34	4182 ± 367[a]	0.12
A-2	338 ± 20	23	3239 ± 586	0.10
A-3	253 ± 8	17	2285 ± 469	0.11

[a] Significantly different from the chloroform–methanol extract.

tions of mussel tissue with chloroform–methanol removed essentially all domoic acid, whereas three or more extractions were necessary to obtain a comparable recovery of domoic acid with either water or acid (Table I). In fact, recovery of domoic acid in acid extract was 74%, compared with recoveries of 94% in water and 91% in chloroform–methanol. This reduced recovery of domoic acid by HCl extraction may be due to some destruction of domoic acid in acidic conditions

TABLE II

RECOVERY OF DOMOIC ACID AT DIFFERENT STEPS OF PURIFICATION FROM BLUE MUSSELS AND PHYTOPLANKTON

Results are expressed as mean ± S.D. from two independent extractions.

Step of purification	Recovery[a] (%)	
	Mussel hepatopancreas	Phytoplankton
Lipid extraction	94.5 ± 7.8	91.1 ± 4.3
Lyophilization	93.2 ± 6.8	84.6 ± 1.6
Ultrafiltration	63.0 ± 4.3	67.2 ± 4.8
Second lyophilization	46.5 ± 4.5	64.6 ± 1.6
"Pure" HPLC fraction	25.8 ± 0.7	36.5 ± 3.3
"Impure" HPLC fraction	17.3 ± 8.3	13.9 ± 4.4

[a] Original concentrations were 1723 μg/ml for hepatopancreas and 1550 μg/ml for phytoplankton.

[1,11]. Most importantly, the extract of toxic mussel tissue or phytoplankton in organic solvent was relatively free from contamination with protein when compared with either water or acid extracts (Table I). Thus, the chloroform–methanol extract required minimal prepurification prior to analysis by HPLC. HPLC analysis of pooled fractions for domoic acid revealed that fractions 4 and 5 contained mostly isomer of domoic acid, fraction 6 contained a mixture of domoic acid and its isomer, and fractions 7–10 contained pure domoic acid (Fig. 2).

Recovery and purity of domoic acid

The recoveries of domoic acid from blue mussel and phytoplankton are similar, with 25.8% of original domoate from mussel tissue recovered and 36.5% recovered from phytoplankton (Table II). These recoveries would likely be improved by processing larger quantities of material.

The purity of domoic acid was examined by UV–VIS absorption spectra using a diode-array spectrophotometer, amino acid analysis by thin-layer chromatography and HPLC analysis of fluorescent derivatives following FMOC derivatization. The UV–VIS spectrum of domoic acid purified by the procedure described in this paper was identical to the spectra of domoic acid purchased from Diagnostic Chemicals and from Sigma (data not reported).

Domoic acid prepared in our laboratory and those purchased from the commercial sources were subjected to one-dimension TLC, and amino acids were visualized after spraying with ninhydrin. Domoic acid showed a pinkish-blue spot and was free from other amino acids (data not reported).

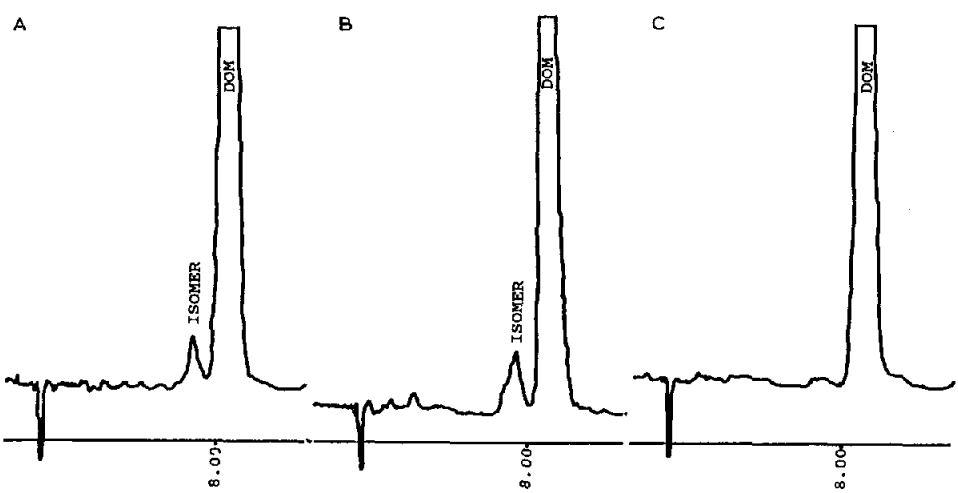

Fig. 3. Analytical HPLC profiles of domoic acid (DOM) purchased from Diagnostic Chemicals (A) or from Sigma (B) and that prepared in our laboratory according to the procedure described in Experimental (C).

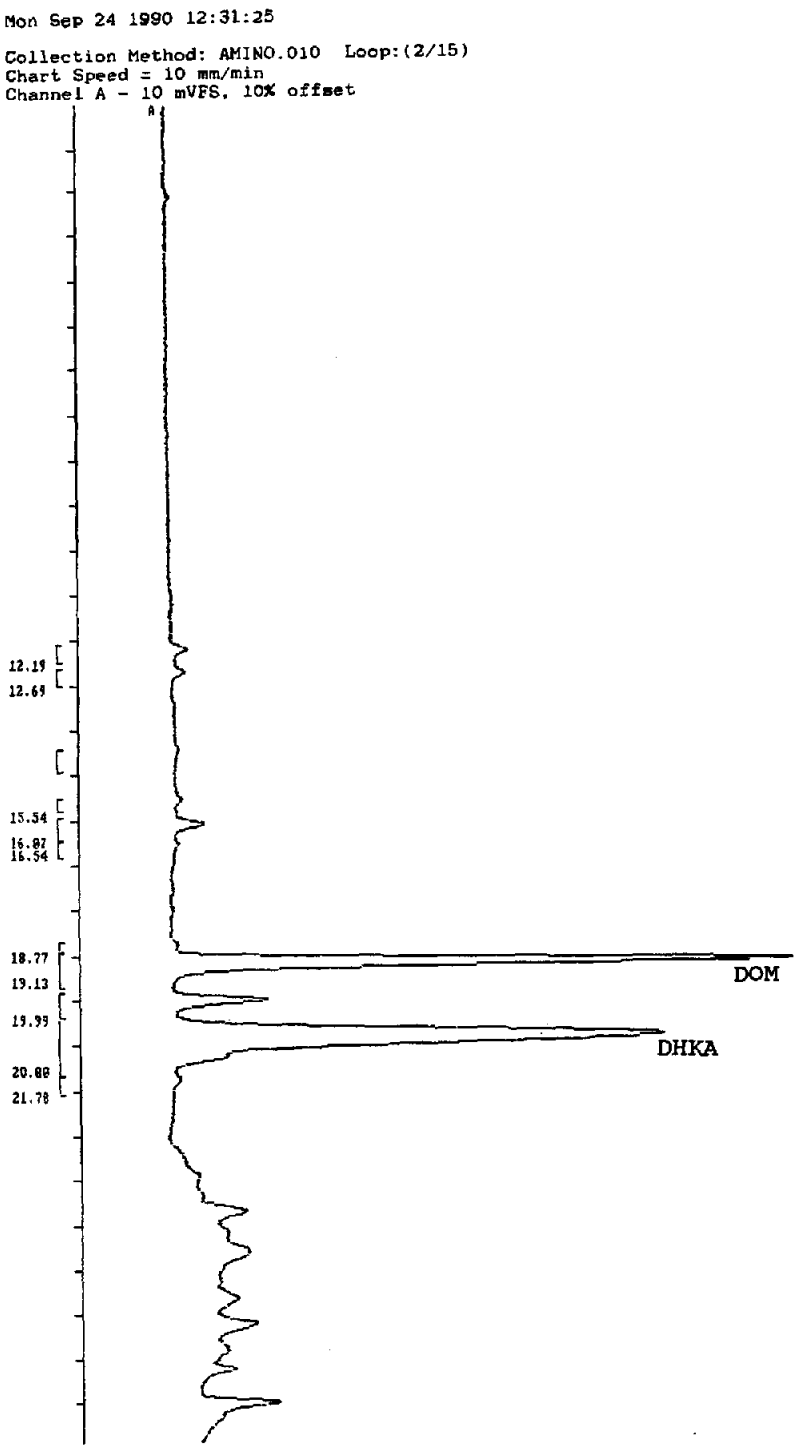

Fig. 4. Preparation of domoic acid by the current method and reaction with FMOC as described in Experimental. The HPLC profile showed two major peaks, one peak with a retention time corresponding to that of primary domoic acid standard (DOM) and the other due to the internal standard, dihydrokainic acid (DHKA). Other minor peaks accounted for less than 2% of pure domoic acid.

TABLE III

TOXICITY OF DIFFERENT PREPARATIONS OF DOMOIC ACID

Fraction 5, 7 and 8 refer to HPLC fractions shown in Fig. 2. Mice were injected with different preparations of domoic acid (150 μg/ml) or its isomer in fraction 5 (200 μg/ml) and times of scratching and death are recorded.

Sample	Concentration (μg/ml)	Weight of mouse (g)	Scratching (min)	Death time (min)
Domoic acid (our preparation)	150	14.4	10	36
		14.7	9	34
		13.8	12	Lived
Diagnostic chemicals	150	17.4	22	35
		17.0	12	17
Fraction 7	150	15.2	13	22
		13.6	14	19
Fraction 8	150	18.3	11	30
		17.7	16	36
		16.0	11	37
Fraction 5	200	16.0	–[a]	–[a]

[a] No response.

Analytical HPLC profiles obtained with domoic acid prepared in this laboratory showed a single peak of domoic acid, whereas domoic acid obtained from Diagnostic Chemicals or Sigma showed small peaks of domoic acid isomer prior to a major peak of domoic acid (Fig. 3).

The results of fluorometric HPLC showed two major peaks, one peak with a retention time comparable to that of domoic acid standard and the other peak due to the internal standard, dihydrokainic acid (Fig. 4), thus confirming the purity of domoic acid.

Biotoxicity of HPLC fractions containing domoic acid

Toxicity of HPLC fractions was measured by the mouse bioassay. Domoic acid-containing fractions showed a strong toxicity, whereas the injection of vehicle into control mice showed no adverse effects. Under identical bioassay conditions, fraction 5 containing only the isomer of domoic acid showed no toxicity (Table III).

DISCUSSION

Results of the present study demonstrate that extraction of either mussel or phytoplankton tissue with a mixture of chloroform–methanol was more effective in extracting domoic acid than extraction of tissues with either water or 0.18 *M* HCl (Table I). Repeated extractions with water or acid were required to achieve a

comparable recovery of domoic acid (Table I). Also, extraction of tissue with acid resulted in a loss of domoic acid. These observations are consistent with the findings of other investigators who reported instability of domoic acid in acidic environment [10,11].

The main features of the procedure described in this paper are that, firstly more than 90% of domoic acid was extracted from the mussel tissue in two extractions, whereas three or more extractions with other solvents were required to accomplish comparable recovery of domoic acid. Secondly, this procedure can also be used effectively to isolate domoic acid from toxic phytoplankton. Thirdly, and most importantly, domoic acid extracted with chloroform and methanol contained much less protein than the extracts with either water or acid (Table I) and, therefore, elaborate clean-up was not necessary to obtain a product which was suitable for preparative HPLC.

The treatment with chloroform and methanol denatured mussel proteins that were located at the interphase between the upper aqueous methanolic and lower chloroform phases, and these were easily removed by filtration. Since large amounts of protein were present in both water and acid extracts (Table I), these extracts required prepurification by solid-gel extraction or ion-exchange chromatography prior to HPLC. In fact, the chloroform–methanol extract was much cleaner than either water or acid extracts which had been subjected to prepurification, which may result in considerable loss of domoic acid. Since the prepurification of chloroform and methanol extract is not necessary, this results in an improved yield of domoic acid. In the current procedure there may be some loss of domoic acid during ultrafiltration through the PM1 filter; however, care with regard to the pressure of nitrogen, patency of Millipore filters and quantitative transfer of domoic acid can essentially eliminate this loss.

Another major advantage of the current procedure is that the adjustment of the pH of the mobile phase, *i.e.* 12.5% acetonitrile in water to pH 3.5 with phosphoric acid, was avoided. This resulted in greatly improved recovery of domoic acid, since the presence of phosphoric acid caused the final preparation of domoic acid to be a gummy yellowish slurry which required repeated crystallization of domoic acid in methanol [1]. Apparently, this step results in a loss of domoic acid. In the present study, we also tested citrate and acetate to adjust the pH of mobile phase to 3.5 in an attempt to avoid the repeated crystallization of domoic acid. In all attempts, a slurry-like preparation of domoic acid resulted. However, when the pH of the mobile phase was not adjusted to 3.5, a powder-like preparation of domoic acid was obtained on lyophilization of pooled fractions.

The purity of domoic acid obtained by the current procedure was examined by analytical HPLC, UV–VIS absorption detection from 190 to 820 nm in a diode-array spectrophotometer, amino acid analysis by TLC and HPLC separation of fluorescent amino acid derivatives, followed by fluorometric detection. By all these criteria domoic acid preparation by the procedure described was as pure as domoic acid available from commercial sources. As well, toxicity of domoic acid

prepared by our method was comparable to domoic acid available commercially. The isomer of domoic acid at 200 μg/ml showed no toxic effects in mice injected intraperitoneally. Domoic acid at a comparable concentration was highly toxic when injected in mice under identical conditions.

In conclusion, the chloroform–methanol procedure for isolation of domoic acid from either toxic mussels or phytoplankton is simple, more effective in extraction of domoic acid, and results in a cleaner preparation than is produced by other methods, even after prepurification with solid-gel and/or ion-exchange chromatography. Thus, extraction with chloroform and methanol improves the recovery of domoic acid, creating a product from which other contaminants can be removed by HPLC. This procedure can be used to isolate domoic acid from both mussel tissue and phytoplankton, and offers good potential for scaling up to isolate large amounts of domoic acid in a cost-effective manner.

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ADDENDUM

The method of domoic acid extraction from blue mussel in a chloroform–methanol mixture was developed by M. S. Nijjar and B. Grimmelt and described in a thesis for M.Sc. degree from the University of Prince Edward Island [5]. This method of extraction was successfully used by our colleagues to estimate domoic acid in rat and guinea pig sera [*J. Chromatogr.*, 526 (1990) 546–549] and may be useful for estimation of domoic acid in other body fluids.

REFERENCES

1 J. L. C. Wright, R. K. Boyd, A. S. W. DeFreitas, M. Falk, R. A. Foxall, W. D. Jamieson, M. V. Laycock, A. W. McCulloch, A. G. McInnes, P. Odense, V. P. Pathak, M. A. Quilliam, M. A. Ragan, P. G. Sim, P. Thibault, J. F. Walter, M. Gilgan, D. J. A. Richard and D. Dewar, *Can. J. Chem.*, 67 (1989) 481.

2 T. M. Perl, L. Bedard, T. Kostasky, J. C. Hockin, E. C. D. Todd and R. S. Remis, *N. Engl. J. Med.*, 322 (1990) 1775.

3 J. S. Teitlebaum, R. J. Zatorre, S. Carpenter, D. Gendron, A. C. Evans, A. Gjedde and N. R. Cashman, *N. Engl. J. Med.*, 322 (1990) 1781.

4 B. Grimmelt, M. S. Nijjar, J. Brown, N. McNair, S. Wagner, G. R. Johnson and J. F. Amend, *Toxicon,* 28 (1990) 501.
5 B. Grimmelt, *M. Sc. Thesis,* University of Prince Edward Island, Charlottetown, 1989.
6 R. Pocklington, J. E. Milley, S. S. Bates, C. J. Bird, A. S. W. DeFreitas and M. A. Quilliam, *Int. J. Environ. Anal. Chem.,* 38 (1990) 351.
7 D. V. Subha Rao, M. A. Quilliam and R. Pocklington, *Can. J. Fish Aquat. Sci.,* 45 (1988) 2076.
8 S. S. Bates, C. J. Bird, A. S. W. De Freitas, R. Foxall, M. W. Gilgan, L. A. Hanic, G. E. Johnson, A. W. McCulloch, P. Odense, R. Pocklington, M. A. Quilliam, P. G. Sim, J. C. Smith, D. V. Subha Rao, E. C. D. Todd, J. A. Walter and J. L. C. Wright, *Can. J. Fish Aquat. Sci.,* 46 (1989) 1203.
9 Association of Official Analytical Chemists, in S. Williams (Editor), *Official Methods of Analysis,* AOAC, Arlington, VA, 14th ed., 1984 p. 344.
10 M. A. Quilliam, P. G. Sim, A. W. McCulloch and A. G. McInnes, *Int. J. Environ. Anal. Chem.,* 36 (1989) 139.
11 J. F. Lawerence, C. F. Charbonneau, C. Menard, M. A. Quilliam and P. G. Sim, *J. Chromatogr.,* 462 (1989) 349.
12 E. G. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.,* 37 (1959) 911.
13 M. M. Bradford, *Anal. Biochem.,* 72 (1976) 248.
14 L. Hanic, University of Prince Edward Island, Charlottetown, personal communication.
15 L. M. Silverman, R. H. Christenson and G. H. Grant, in N. W. Tietz (Editor), *Text Book of Clinical Chemistry,* W. B. Saunders, Philadelphia, PA, 1986, p. 540.