Use of Fluorometry for the Determination of *Gonyaulax tamarensis* var. excavata Toxins in New England Shellfish

The use of a modified fluorometric procedure for the determination of paralytic shellfish poisons in New England shellfish is described. Hot 0.1 M hydrochloric acid extracts used in the standard mouse bioassay are treated with alkaline hydrogen peroxide, and the resulting fluorescence is read. Results on the soft shell clam (*Mya arenaria*) averaged 22% higher than those of the mouse bioassay and results with the mussel (*Mytilus edulis*) averaged 11% higher.

The formation of highly fluorescent derivatives when saxitoxin (STX) and related toxins are oxidized by hydrogen peroxide has been used as the basis for several methods which have been described for the determination of paralytic shellfish poisons (PSP) by chemical means (Bates and Rapoport, 1975; Bates et al., 1978; Buckley et al., 1976, 1978).

In this paper we wish to report the results of a modification of the abbreviated Bates and Rapaport (1975) method.

EXPERIMENTAL SECTION

Shellfish Samples. Samples which had been collected during the 1972 New England Red Tide and stored in the frozen state as well as freshly collected samples were used. All samples were collected from the New Hampshire and the adjoining northern Massachusetts area.

Saxitoxin Standard. A U.S. Public Health Service reference standard containing 100 μg of STX/mL was used to standardize the mouse assay and the fluorometric assay.

Mouse Bioassay. Bioassays were carried out by using the standard AOAC method (Horwitz, 1965) with mouse strains $B6D_2F_1/J$ or C57BL/6J (Jackson Laboratories, Bar Harbor, ME) or strain CD-1 (Charles River Breeding Laboratories (Wilmington, MA). Some samples had been bioassayed by the State of New Hampshire Division of Public Health Services in Concord, NH, as part of its routine monitoring service for PSP in shellfish in the State.

Fluorometric Tube Assay Procedure. The modified Bates and Rapoport method developed by Shoptaugh (1978) is as follows. (1) The extract to be assayed was prepared in the same manner as in the mouse assay, i.e., 100 g of shellfish meat was homogenized in a blender with 100 mL of 0.1 N HCl, and the mixture was boiled gently for 5 min, cooled to room temperature, brought to pH 4.0–4.5 with HCl or NaOH, and brought to a volume of 200 mL with water. The mixture was then centrifuged in a clinical centrifuge for 5 min. (The extraction procedure could be scaled down.) (2) Aliquots of the supernatant were removed (2-mL maximum) and diluted to 2 mL with water. A series of blanks containing the same amounts of supernatant was also prepared. (3) Two milliliters of 1 N NaOH was added to each tube. (4) To each sample tube 0.6 mL of 1% H_2O_2 was added and the contents were mixed with a vortex stirrer. To each blank tube 0.6 mL of water was added. (5) All tubes were incubated in the dark at room temperature for 40 min. (6) To each tube was added 0.2 mL of glacial acetic acid. (7) The fluorescence was read in a Farrand MK-1 spectrofluorometer with excitation at 330 nm and a 5-nm slit width and emission at 380 nm and a 10-nm slit width. (8) From the fluorescence of each sample tube, the fluorescence of the corresponding blank was subtracted, and the corrected fluorescence (microamperes) was plotted vs. volume of extract. If the curve was not linear, smaller volumes of extracts were used. (9) For calculation of the microgram of STX equivalents per milliliter of extract, the μA/mL slope of the curve was divided by the μA/μg slope of a standard curve obtained with STX (0.05–1.5 μg of STX). (10) When the Raman peak (~0.002 μA) at 370 nm of pure water (excitation at 330 nm) was used to calibrate the system, it corresponded to 0.015 μg of STX in 2 mL of sample. Bates and Rapoport (1975) reported that the Raman peak corresponded to 0.017 μg of STX/g of shellfish (in 2 mL of extract).

RESULTS AND DISCUSSION

The results of the use of the modification of the abbreviated Bates and Rapoport method are shown in Table I. There is a general agreement between the results of the fluorometric assay with those of the mouse bioassay, with the results averaging 22 ± 10% higher than those of the mouse assay in the case of *Mya* and 11% ± 8% higher in the case of *Mytilus*. Since the mouse bioassay result is normally based on three mice and is subject to a standard error of the mean of ±20% and since low levels of toxicity (~80 μg/100 g of shellfish meat) may be underestimated by as much as 60% (Schantz et al., 1958), it is difficult to assess the true accuracy of the present method.

Use of the Bates and Rapoport method for determining saxitoxin has been disappointing when applied to the determination of PSP in general (Taylor and Seliger, 1979). Bose and Reid (1979), using the column procedure, report a 14–38% recovery of STX, as based on the mouse assay, in butter clam and mussel samples collected in British Columbia, Canada, and attribute the discrepancies to the presence of PSP's other than STX. White and Maranda (1978), using both the column method and the abbreviated method, report values for *Gonyaulax excavata* cells and for *Mya arenaria*, *Mytilus edulis*, and *Modiolus modiolus* collected in the New Brunswick, Canada, area which were 3–48% with the column and 14–73% without the column of the mouse bioassay values. These low values were consistent with the idea that the column method was detecting only STX and that the abbreviated method, although it was detecting STX and additional toxins, was still not detecting all of the toxins which were being detected by the mouse bioassay.

The *Gonyaulax tamarensis* var. excavata [name proposed by Schmidt and Loeblich (1979) for the New England organism to replace *G. excavata*] toxins vary widely in their fluorescence yield per mouse unit (MU), with GTX_1 and neo-STX giving very low yields and GTX_5 giving a very high yield when compared with STX (Buckley et al., 1978). It might, therefore, appear that a fluorometric analysis may be inapplicable unless the individual toxins could be estimated. However, the principal toxins in toxic New England shellfish appear to be GTX_2, GTX_3, and STX, all of which give similar fluorescence

0021-8561/81/1429-0198$01.00/0

Table I. Comparison of the Fluorometric Method with the Mouse Bioassay in the Determination of PSP Levels in Shellfish

shellfish	μg of PSP (as STX)/100 g of shellfish meat: mouse bioassay[a]	fluorometric method	% difference from the mouse bioassay[b]
M. arenaria	3950 (3212)	3080	−22
	3950 (3212)	2124	−46
	2161	2124	−2
	(2072)	2030	(−2)
	(1063)	1145	(+8)
	1043 (1725)	1569	+50
	622 (801)	803	+29
	622 (801)	786	+26
	550 (554)	620	+13
	550 (554)	647	+18
	452 (381)	730	+62
	452 (381)	404	−11
	(414)	517	(+25)
	300	414	+38
	232	398	+72
	171	273	+60
	<40	39	
	<40	12	
	<40	0	
			av (*Mya*): 22.1 ± 9.8[c]
M. edulis	1472 (1443)	1332	−10
	1472 (1443)	1316	−11
	1142	1031	−10
	735 (907)	952	+30
	735 (907)	702	−4
	561	345	−39
	456	214	−53
	259	380	+47
	156	128	−18
	95	130	+37
	86	101	+17
	73	69	−5
	71	92	+30
	63	113	+79
	46	54	+17
	43	66	+53
	40	48	+20
	<40	43	
	<40	26	
	<40	21	
			av (*Mytilus*): 10.6 ± 8.3
Spissula solidissima	40	63	+58

[a] Age of samples varied from fresh to 7 years in the frozen state. In all cases (except three) samples were bioassayed at the time the fluorometric assays were run. 1972 assay figures (in parentheses) are compared with 1979 assay figures for the same sample. [b] Values in parentheses are based on 1972 bioassay figures. [c] Does not include values in parentheses.

yields (Buckley et al., 1976, 1978). Therefore, unless significant amounts of GTX_1, neo-STX, and GTX_5 are present in these shellfish samples, an approximate value for PSP levels may be obtained by fluorometry. This seems to be the case with the New England shellfish that we have examined, where we have obtained recoveries of activity of 54–172% with *M. arenaria* and 47–179% with *M. edulis*, these values being calculated by adding 100 to the percent difference from the mouse bioassay in Table I. Part of this large range might be explained by the inherent inaccuracy of the mouse bioassay, upon which our comparisons are based. In addition, the presence of any GTX_1 and neo-STX would tend to reduce the fluorometric value and any GTX_5 would tend to increase it. Since the fluorometric method appears to be more sensitive than the mouse assay for the detection of PSP's, it may be a useful procedure for detecting trends in PSP activity before they can be detected by the mouse assay.

ACKNOWLEDGMENT

We thank the State of New Hampshire Division of Public Health Services for supplying samples which had been assayed by the mouse assay during shellfish monitoring for PSP within the State.

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Received for review July 23, 1980. Accepted October 7, 1980. The method is from a dissertation submitted by the senior author in 1978 to the Graduate School, University of New Hampshire, Durham, in partial fulfillment of the requirements for the Ph.D. degree. This work was supported in part by Hatch Project 205 of the New Hampshire Agricultural Experiment Station and by a grant from the U.S. Department of Commerce Office of Sea Grant. Published as Scientific Contribution No. 1045 of the New Hampshire Agricultural Experiment Station and University of New Hampshire Sea Grant Publication No. UNH-SG-JR-129.

Halogen Compounds Identified in the Volatile Constituents of Baked Potatoes

The volatile constituents of baked potatoes were isolated from 540 lb of Idaho Russet Burbank baked potatoes. The components of the baked potato volatiles were separated into relatively pure fractions by repetitive gas chromatography. The fractions were then analyzed by GC–MS and infrared spectometry. Fourteen halogenated compounds were identified.

Residues of halogenated compounds used as pesticides, herbicides, and fungicides found in food is a topic which might be of vital importance to the health of consumers. Organochlorine pesticides were found to be persistent in the soil (Brown, 1978). The half-life in the soil for heptachlor, chlordane, and lindane was ~1 year, and for dieldrin, endrin, and DDT the half-life was in excess of 2 years (Edwards, 1973). Residues of heptachlor, aldrin, and dieldrin were found in various crops including soybeans (Eden and Arthur, 1965; Bruce and Decker, 1966) and potatoes (Terriere and Ingalsbe, 1953). The residual 2,2-dichloropropionic acid, a herbicide, was found in citrus fruits (Getzendaner et al., 1965). 1,2-Dibromo-3-chloropropane is used as a soil fumigant. Residues of 1,2-dibromo-3-chloropropane were found in beans, in potatoes (Guinn and Potter, 1962), in oranges (Castro and Schmitt, 1962), and on grains (Fishbein, 1976). The use of ethylene dichloride and ethylene dibromide in fumigant mixtures of disinfecting fruits, vegetables, food grains, and tobacco suggests the possibility that their residues or their respective hydrolytic products may be present in fumigated material (Beck, 1974; Fishbein, 1976; Dumas, 1973). The accumulation of organochlorine pesticides in poultry has been extensively studied (Kan, 1978). Organochlorine pesticides were found to be persistent, and residues can accumulate in fat and eggs (Kan, 1978). In general, it can be said that halogen residues are persistent and undesirable in animal and plant products because of their possible toxic properties.

Although the use of many chlorinated hydrocarbon insecticides has been banned, some halogenated insecticides and fungicides are still being used on crops. For example, thiodan and phosphamidon are recommended in Idaho to be used to control the Colorado potato beetle (Homan et al., 1979) and ethylene dibromide is used as fumigant to control wireworm in potato (Sandvol et al., 1978); also, preplant treatment of pentachloronitrobenzene is used to control the rhizoctonia disease of potato (Davis, 1977). In addition, pentachloronitrobenzene is also used to control the common scab of potato (Davis and Garner, 1978).

In our study of the volatile flavor compounds of baked potatoes, we identified 14 halogenated compounds. The present paper reports their identification.

EXPERIMENTAL SECTION

The details of experimental procedures were described in Coleman et al. (1981).

Table I. Halogen Compounds Identified in the Volatile Constituents of Baked Potatoes

no. of peaks[a]	identified as	mass fragmentation, *m/e* (%)
1-5-2	1,1,1-trichloroethane	97 (100), 99 (71), 117 (28), 61 (24), 119 (18), 101 (15), 63 (11), 62 (8)
1-5-3	tetrachloroethylene	166 (100), 164 (81), 129 (58), 131 (55), 168 (47), 94 (30), 133 (22), 47 (18)
1-6-5	trichloroacetic acid	44 (100), 85 (75), 83 (52), 36 (38), 35 (22), 47 (20), 38 (16), 87 (11)
2-1-2	2-chloropropane	43 (100), 63 (30), 27 (21), 78 (20), 41 (18), 80 (10), 65 (6), 39 (6)
2-12-4	chloroform	83 (100), 85 (67), 47 (35), 49 (20), 35 (10), 48 (9), 50 (7), 37 (5)
3-8	1-chloroheptane	91 (100), 43 (50), 55 (49), 41 (40), 69 (31), 93 (29), 57 (25), 56 (18)
4-3	1,1-dichloroheptane	70 (100), 43 (60), 41 (47), 55 (42), 27 (38), 67 (22), 81 (20), 29 (19)
4-4	1-chloro-2-methylbutane	70 (100), 43 (95), 41 (90), 55 (71), 42 (65), 27 (45), 29 (40), 39 (35)
10-13	*o*-chloroaniline	127 (100), 65 (37), 129 (35), 92 (30), 128 (25), 91 (22), 64 (17), 63 (15)
10-25	2-chlorobiphenyl	188 (100), 152 (33), 153 (32), 190 (30), 189 (20), 76 (18), 151 (15), 63 (10)
11-6	2-bromo-5-ethylnonane	57 (100), 55 (60), 43 (59), 69 (44), 41 (32), 71 (28), 97 (18), 85 (16)
11-12	*p*-chloroaniline	127 (100), 65 (35), 129 (32), 92 (25), 39 (15), 63 (12), 100 (12), 128 (10)
12-6	1-iodooctadecane	57 (100), 71 (72), 43 (52), 85 (50), 41 (42), 55 (32), 69 (27), 99 (18)
13-7	1-chlorohexadecane	57 (100), 43 (90), 71 (56), 41 (49), 91 (39), 55 (22), 85 (18), 69 (16)

[a] The first, second, and third numerals indicate the number of gas chromatographic peaks during the original broad gas chromatographic fractionation, the second chromatography, and the third chromatography, respectively.

0021-8561/81/1429-0200$01.00/0