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Distribution and Isolation of 2－aminoethylphosphonic acid (2－AEP) of the Edible Shellfishs.

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ABSTRACT

This investigation was carried out in order to demonstrate the occurrence of carbon-phosphorus compound (2-AEP) in the sixteen species of edible shellfishs.

Edible shellfishs had previously been shown to contain of 2-AEP, the present study has added a number of new edible shellfishs to the positive list.

2-AEP has been found large amount in chloroform-methanol soluble (lipid) fraction and in the TCA-insoluble fraction.

About twenty-one mg, nine mg, five mg and two mg of the 2-AEP were isolated from the lipid of the TENGUNISHI, *Hemifusus ternatanus*, UCHIWAEBI, *Ibacus ciliatus*, GAZAMI, *Portunus trituberculatus* and UNI, *Hygrosoma hoplacantha*, respectively.

The compound was purified with a combination of ion exchange column chromatography with Dowex 50-X 4 and Dowex 1-X 8 resins. The infrared spectrum of the isolated compound showed an absorption band at 1180 cm^{-1} due to C-P bond, and was essentially identical with that of the authentic 2-AEP.

Upon hrdrolysis of the compound by strong acid, neither change in the chromatographic behaviors of this compound nor liberation of inorganic phosphate was observed.

The stability of the compound to acid hydrolysis suggested the presence of a C-P bond.

On comparison with synthetic compound, the aqueous hydrolysis product behaved like 2-aminoethylphosphonic acid on thin layer chromatogram.

It has been demonstrated that the sixteen species of edible shellfish used here contains high concentration of the 2-AEP.

INTRODUCTION

The amino acid, 2-aminoethylphosphonic acid was first isolated by Horiguchi and Kandatsu[1] from the lipid fraction of rumen ciliate protozoa. Since the isolation of 2-AEP, twenty-nine other C-P compounds structurally related to 2-AEP have been discovered in biological materials[2]. 2-AEP occurs in the bound form to proteins and lipids as well as in the free form and some-times accounts for as much as 50 % of the total phosphorus of the organism[3]. In view of the previously mentioned incorporation studies in animals, there is the possibility of the widespread occurrence of 2-AEP among mammalian animals.

Therefore, our interest in mammalian metabolism of 2-AEP has led to the present investigation on the detection and isolation of the 2-AEP from shellfishs which is well known as a suitable food for human.

MATERIALS AND METHODS

Materials. Sixteen species of edible shellfishes were purchased on the local market. 2-AEP was prepared by the method described by kosolapoff[4] and were purified by column chromatography and recrystallization. The resins employed were Dowex 50-X4 and Dowex 1-X8, 200-400 mesh, supplied by Dow Chemical Laboratories, U. S. A. The resins were purified by the method of previous report[5].

All reagents used were either of analytical grade or of the highest purity available.

Thin-layer chromatography. The chromatographic separation was carried out on thin-layer plate of silica gel F_{254} Merck 0.25 mm thick. The following solvent systems were used :

1. Ethanol : 70 % ammonia (1 : 2, v/v)
2. Isopropanol : acetic acid : 15 % ammonia : water (5 : 2 : 4 : 3, v/v)
3. Methanol : formic acid : water (16 : 3 : 1, v/v)
4. 0.02N acetic acid in 60 % ethanol
5. Methanol : pyridine : water (20 : 1 : 5, v/v)

Reagents for detection. The ninhydrin reagent was 0.2 % (w/v) in acetone. The phosphonates was detected with the dipreagent developed by Rosenberg[6] for orthophosphate and phosphate esters. Phosphonates yield transient green to blue-green spots with this reagent.

Phosphorus analysis. Inorganic phosphate was estimated by the method of Chen et al[7]. Total phosphorus was determined as inorganic phosphate after ashing of

the samples with 6 N H_2SO_4 for about 3 hr and with three drops of perchloric acid for an hour. Phosphonate phosphorus was estimated by the method of Tamari et al[8].

Melting point and infrared spectra. Melting point and infrared spectra were determined as described previously[8].

Fractionation of phosphorus fractions in edible shellfish.

The freeze dried materials were extracted twice with 40 ml of acetone by an efficient magnetic stirrer in a 100 ml beaker at room temperature. The acetone extracts was centrifuged at 3000 rpm for 15 min. The supernatants was evaporated to dryness under reduced pressure (A fraction).

The residue was extracted four times with 50 ml of chloroform-methanol (2 : 1, v/v) for 30 min. at 40°C, and centrifuged at 3000 rpm for 15 min. The four combined extracts were evaporated to dryness under reduced pressure (B fraction). The residue was suspended in 50 ml of 5 % trichloroacetic acid and the suspension was stirred at 40°C for 30 min. The suspension was then centrifuged for 15 min. at 3000 rpm. The precipitate was re-extracted twice under the same conditions. The combined supernatant was evaporated to dryness under reduced pressure (C fraction), and the precipitate (D fraction) was with 30 ml of ether, ethanol, and hot ethanol successively.

Extraction and hydrolysis of the lipids.

The lipid of the freeze dried sample (GAZAMI, UNI, UTIWAEBI and TENGUNISHI were used 16, 32, 55 and 134 g for analysis) was extracted by the method as previously described[5].

The lipid extract was hydrolysis according to the method as previously described[5].

Isolation and purification of 2-AEP in the lipid hydrolysate.

The 2-AEP in the lipid hydrolysate was isolated with a combination of Dowex 50-X4, Dowex 1-X8 and thin layer chromatography according to the method as previously described[5].

RESULTS AND DISCUSSION

1. *Total phosphorus and phosphonate-phosphorus in the four fractions of edible shellfishs.*

A portion of the A, B, C, D fraction was hydrolysed with 6 N HCl as described in the experimental method. Water was added to the hydrolysate, and the mixture

Table. 1 Contents of C-P and T-P in the four fractions of Edible Shellfish. (No. 1)

Japanese name	Scientific name	Fraction	C-P (mg/100g) %	T-P (mg/100g)	C-P/T-P (%)
Hamaguri	Meretrix	A	0.270 (5.11)	3.221	8.39
	Iusoria	B	2.680 (50.73)	84.607	3.17
		C	0.528 (9.99)	20.302	2.60
		D	1.805 (34.17)	163.682	1.10
		total	5.283	275.033	1.92
Sijimi	Carbicula	A	3.653 (6.92)	54.783	6.67
	japonica	B	10.375 (19.66)	40.379	25.70
		C	0.374 (0.71)	12.114	3.09
		D	38.383 (72.72)	376.315	10.20
		total	52.784	483.590	10.92
Asari	Venerupis	A	0.125 (0.22)	9.119	1.37
	philippinamm	B	20.147 (35.28)	90.274	22.32
		C	0.907 (1.59)	31.183	2.91
		D	35.297 (62.91)	366.700	9.80
		total	57.106	497.277	11.48
Akagai	Scapharca	A	0.140 (1.67)	5.123	2.74
	broughtonii	B	5.604 (66.97)	89.285	6.28
		C	0.194 (2.32)	21.799	0.89
		D	2.430 (29.04)	182.094	1.33
		total	8.368	293.302	2.81
Sazae	Turbo cornutus	A	0.017 (0.39)	1.973	0.87
		B	1.615 (36.95)	122.893	1.31
		C	0.542 (12.40)	36.547	1.48
		D	2.197 (50.26)	177.205	1.24
		total	4.371	338.619	1.29
Baigai	Babylonia	A	0.118 (1.28)	0.171	68.57
	japonica	B	1.716 (18.76)	88.096	1.95
		C	0.704 (7.70)	36.419	1.93
		D	6.611 (72.27)	188.657	3.50
		total	9.148	313.344	2.92

( ) : as % of total C-P

Continue to No. 2

Table. 1 Contents of C—P and T—P in the four fractions of Edible Shellfish. (No. 2)

Japanese name	Scientific name	Fraction	C-P (mg/100g) %	T-P (mg/100g)	C-P/T-P (%)
Kurumaebi	Penaeus	A	0.149 (0.79)	4.448	1.47
	japonicus	B	1.641 (8.68)	63.502	4.94
		C	7.943 (42.04)	277.065	0.43
		D	9.162 (48.49)	152.617	2.64
		total	18.895	497.632	3.80
Amaebi	Pandalus	A	0.000 (0.00)	5.456	0.00
	borealis	B	0.727 (23.42)	18.664	3.90
		C	0.000 (0.00)	30.187	0.00
		D	2.377 (76.58)	47.464	5.00
		total	3.104	101.770	3.50
Akaebi	Metapenaeopsis	A	2.023 (38.67)	3.714	54.41
	borbata	B	0.803 (15.34)	50.654	1.59
		C	0.146 (2.79)	21.725	0.75
		D	2.260 (43.18)	79.229	2.85
		total	5.234	155.323	3.37
Shako	Squilla	A	1.561 (42.88)	56.482	2.77
	oratoria	B	1.494 (41.04)	65.636	2.28
		C	0.328 (9.01)	39.451	0.83
		D	0.257 (7.06)	31.786	0.81
		total	3.640	193.155	1.88
Awabi	Haliotis discus	A	107.124 (46.17)	371.934	28.80
		B	12.462 (5.37)	85.358	14.60
		C	0.043 (0.02)	30.257	0.14
		D	112.388 (48.44)	465.600	24.14
		total	232.018	953.148	24.34

( ) : as % of total C—P

Continue to No. 3

Table. 1 Contents of C-P and T-P in the four fractions of Edible Shellfish. (No. 3)

Japanese name	Scientific name	Fraction	C-P (mg/100g) %	T-P (mg/100g)	C-P/T-P (%)
Utiwaebi	Ibacus ciliatus	A	0.049 (0.12)	14.266	0.34
		B	5.758 (14.61)	27.640	20.83
		C	1.135 (2.88)	30.012	2.27
		D	32.461 (82.38)	123.259	26.34
		total	39.405	215.176	18.26
Tengunishi	Hemifusus ternatanus	A	0.437 (3.14)	9.710	4.51
		B	7.337 (52.74)	39.342	18.65
		C	0.447 (3.21)	17.603	2.54
		D	5.690 (40.90)	126.693	4.49
		total	13.911	193.349	7.19
Nina	Batillaria multiformis	A	0.316 (2.76)	12.353	2.56
		B	11.032 (96.47)	44.534	24.77
		C	0.046 (0.40)	31.333	0.15
		D	0.041 (0.36)	108.656	0.04
		total	11.436	196.878	5.81
Gazami	Portunus trituberculatus	A	0.848 (10.13)	30.157	2.81
		B	3.836 (46.26)	297.750	1.29
		C	1.956 (23.59)	405.779	0.48
		D	1.659 (20.00)	66.673	2.49
		total	8.293	800.359	1.04
Uni	Hygrosoma hoplacantha	A	1.405 (16.71)	53.001	3.18
		B	2.148 (25.55)	205.243	1.26
		C	4.288 (51.01)	944.597	0.65
		D	0.566 (6.73)	188.808	0.30
		total	8.407	1391.649	6.04

( ) : as % of total C—P

was extracted several times with ether. The aqueous layer was filtered, evaporated to dryness, and redissolved with small amounts of water for phosphorus analysis. The results of C-P and T-P analysis in the A, B, C, and D fractions of the edible shellfishs are shown in Table 1.

The amount of C-P in the A, B, C and D fractions were 0.27, 2.68, 0.53 and 1.81 mg per 100 g of HAMAGURI, respectively. The B fraction contained 50.7 % of the C-P in the HAMAGURI, and the amount of C-P of the B fraction was about 10, 5 and 1.5 times higher than in the A, C and D fraction.

The amount of C-P in the A, B, C and D fractions were 3.65, 10.38, 0.37 and 38.38 mg per 100 g of SIJIMI, respectively. The D fraction contained 72.7 % of the C-P in the SIJIMI, and the amount of C-P of the D fraction was about 10, 4 and 103 times higher than in the A, B and C fraction.

The amount of C-P in the A, B, C and D fractions were 0.13, 20.15, 0.90 and 35.30 mg per 100 g of ASARI, respectively.

The D fraction contained 62.9 % of the C-P in the ASARI, and the amount of C-P of the D fraction was about 286, 1.8 and 40 times higher than in the A, B and C fraction.

The amount of C-P in the A, B, C and D fractions were 0.14, 5.60, 0.19 and 2.43 mg per 100 g of AKAGAI, respectively.

The B fraction contained 67.0 % of the C-P in the AKAGAI, and the amount

of C-P of the B fraction was about 40, 29 and 2.3 times higher than in the A, C and D fraction.

The amount of C-P in the A, B, C and D fractions were 0.02, 1.62, 0.54 and 2.20 mg per 100 g of SAZAE, respectively.

The D fraction contained 50.3 % of the C-P in the SAZAE, and the amount of C-P of the D fraction was about 128, 1.4 and 4 times higher than in the A, B and C fraction.

The amount of C-P in the A, B, C and D fractions were 0.12, 1.72, 0.70 and 6.61 mg per 100 g of BAIGAI, respectively.

The D fraction contained 72.3 % of the C-P in the BAIGAI, and the amount of C-P of the D fraction was about 56, 3.9 and 9.4 times higher than in the A, B and C fraction.

The amount of C-P in the A, B, C and D fractions were 0.15, 1.64, 7.94 and 9.16 mg per 100 g of KURUMAEBI, respectively.

The D fraction contained 48.5 % of the C-P in the KURUMAEBI, and the amount of C-P of the D fraction was about 61, 5.6 and 1.2 times higher than in the A, B and C fraction.

The amount of C-P in the A, B, C and D fractions were 0.0, 0.73, 0.0 and 2.38 mg per 100 g of AMAEBI, respectively.

The D fraction contained 76.6 % of the C-P in the AMAEBI, and the amount of C-P of the D fraction was about 76, 3.3 and 76 times higher than in the A, B and C fraction.

The amount of C-P in the A, B, C and D fractions were 2.02, 0.83, 0.15 and 2.26 mg per 100 g of AKAEBI, respectively.

The D fraction contained 43.2 % of the C-P in the AKAEBI, and the amount of C-P of the D fraction was about 1.1, 2.8 and 15 times higher than in the A, B and C fraction.

The amount of C-P in the A, B, C and D fractions were 1.56, 1.49, 0.33 and 0.26 mg per 100 g of SHAKO, respectively.

The A fraction contained 42.9 % of the C-P in the SHAKO, and the amount of C-P of the A fraction was about 1.0, 4.8 and 6.0 times higher than in the B, C and D fraction.

The amount of C-P in the A, B, C and D fractions were 107.12, 12.46, 0.04 and 112.39 mg per 100 g of AWABI, respectively. The D fraction contained 48.44 % of the C-P in the AWABI, and the amount of C-P of the D fraction was about 1.0, 9.0 and 2422 times higher than in the A, B and C fraction.

The amount of C-P in the A, B, C and D fractions were 0.05, 5.76, 1.14 and 32.46 mg per 100 g of UTIWAEBI, respectively.

The D fraction contained 82.4 % of the C-P in the UTIWAEBI, and the amount of C-P of the D fraction was about 687, 5.6 and 29 times higher than in the A,

B and C fraction.

The amount of C-P in the A, B, C and D fractions were 0.44, 7.34, 0.45 and 5.70 mg per 100 g of TENGUNISHI, respectively.

The B fraction contained 52.7 % of the C-P in the TENGUNISHI, and the amount of C-P of the B fraction was about 17, 16 and 1.3 times higher than in the A, C and D fraction.

The amount of C-P in the A, B, C and D fractions were 0.32, 11.03, 0.05 and 0.04 mg per 100 g of NINA, respectively.

The B fraction contained 96.5 % of the C-P in the NINA, and the amount of C-P of the B fraction was about 35, 241 and 268 times higher than in the A, C and D fraction.

The amount of C-P in the A, B, C and D fractions were 0.85, 3.84, 1.96 and 1.66 mg per 100 g of GAZAMI, respectively.

The B fraction contained 46.3 % of the C-P in the GAZAMI, and the amount of C-P of the B fraction was about 4.6, 2.0 and 2.3 times higher than in the A, C and D fraction.

The amount of C-P in the A, B, C and D fractions were 1.41, 2.15, 4.29 and 0.57 mg per 100 g of UNI, respectively.

The C fraction contained 51.0 % of the C-P in the UNI, and the amount of C-P of the C fraction was about 3.0, 2.0, and 7.6 times higher than in the A, B and D fraction.

2. *Isolation and purification of 2-AEP in the lipid.*

Fig. 1 shows the elution pattern on a Dowex 50-X 4 column (2.5 x 26 cm) of organophosphorus in the acid hydrolysate of the lipid from TENGUNISHI, *Hemifusus ternatanus*.

Tube No. 8～15, 24～28, 31～39 and 52～57 inclusive contained ninhydrine-and Rosenberg reagent-positive material on thin layer chromatogram. The fraction between tube No. 31 and 39 of the eluate was rich in phosphorus, and the phosphorus containing substance were found to consist predominantly 2-AEP by thin layer chromatography.

This crude material of tube No. 31～39 in the Fig. 1 was further purified on a Dowex 50-X 4 column (1 x 50 cm) with 0.6N HCl (Fig. 2), still contained a small amount of contaminants, and was therefore purified by a third passage through the Dowex 1-X 8 column with 0.5N acetic acid.

After the removal of acetic acid the residue was dissolved in 0.5 ml of water, and ethanol was added to 50 % concentration.

Recrystallization three times from the same solvent yielded 21 mg of rhombic crystals which melted with decomposition at 280～281°C.

Anal. Found : C, 19.9 ; H, 6.1 ; N, 11.3 ; P, 25.6.

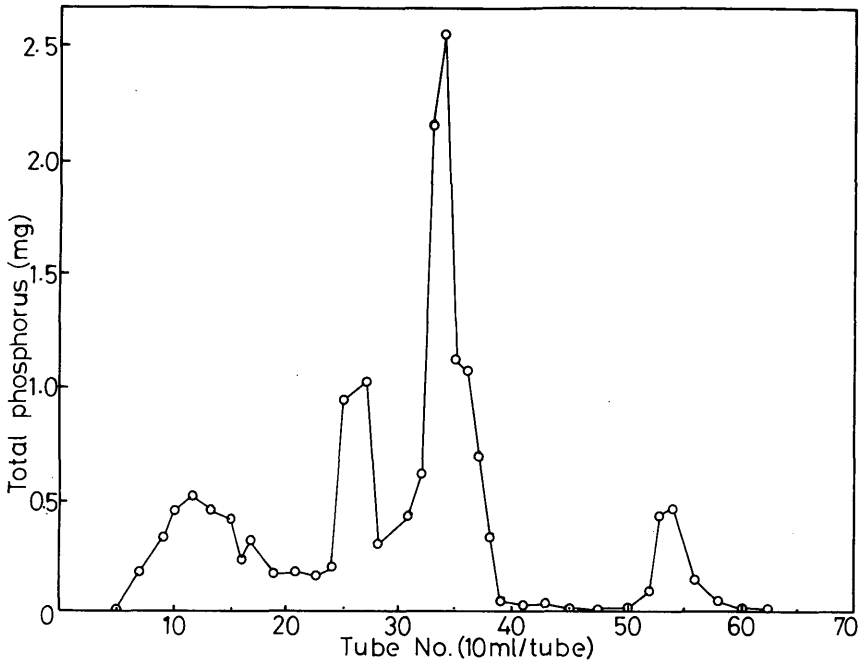

Fig. 1. Elution pattern on a Dowex 50-X 4 column (2.5 x 26 cm) of organophosphorus in the acid hydrolysate of the lipid from TENGUNISHI, *Hemifusus ternatanus*.

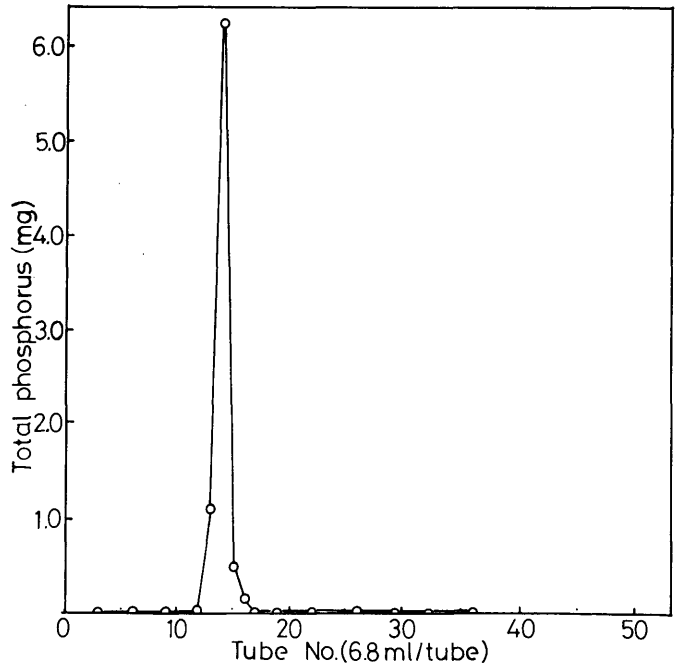

Fig. 2. Rechromatographic elution pattern on a Dowex 50-X 4 column (1 x 50 cm) of the peak (tube No. 31～39) in the Fig. 1.

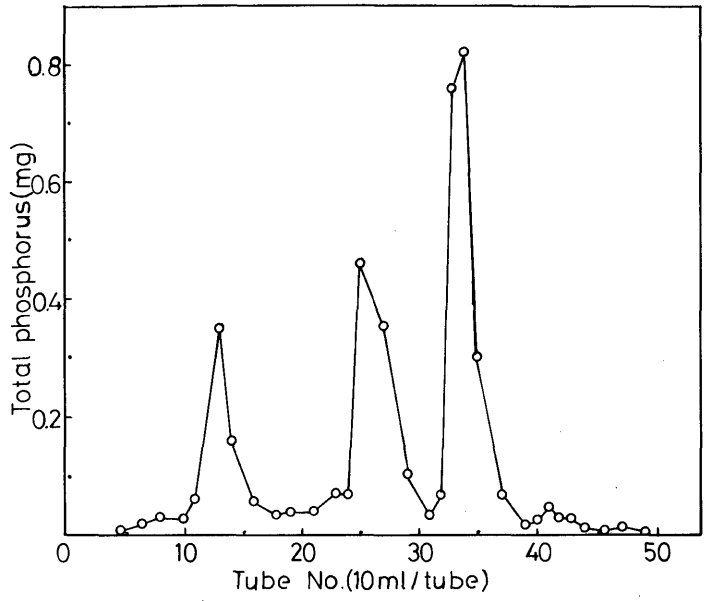

Fig. 3. Elution pattern on a Dowex 50-X 4 column (2.5 x 60 cm) of organophosphorus in the acid hydrolysate of the lipid from UCHIWAEBI, *Ibacus ciliatus*.

Calcd. for $C_2H_8NPO_3$: C, 19.2 ; H, 6.4 ; N, 11.2 ; P, 24.8.

Fig. 3 shows the elution pattern on a Dowex 50-X 4 column (2.5 x 60 cm) of organophosphorus in the acid hydrolysate of the lipid from UCHIWAEBI, *Ibacus ciliatus*.

The tube No. 10～16, 24～30 and 32～38 inclusive contained ninhydrine-and Rosenberg reagent-positive material on thin layer chromatogram. The fraction between tube No. 24 and 30 of the eluate was rich in phosphorus, and the phosphorus containing substance were found to consist predominantly 2-AEP by thin layer chromatography. This crude material of tube No. 24～30 in the Fig. 3 was further purified on a Dowex 50-X 4 column (1.0 x 50 cm) with 0.6N HCl (Fig. 4), still contained a small amount of contaminants, and was therefore purified by a third passage through the Dowex 1-X 8 column with 0.5N acetic acid.

After the removal of acetic acid the residue was dissolved in 0.5 ml of water, and ethanol was added to 50 % concentration.

Recrystallization three times from the same solvent yielded 9.3 mg of rhombic crystals which melted with decomposition at 280～281°C.

Anal. Found : C, 19.3 ; H, 6.3 ; N, 11.4 ; P, 25.2.

Calcd. for $C_2H_8NPO_3$: C, 19.2 ; H, 6.4 ; N, 11.2 ; P, 24.8.

Fig. 5 shows the elution pattern on a Dowex 50-X 4 column (2 x 30 cm) of organophosphorus in the acid hydrolysate of the lipid from GAZAMI, *Portunus*

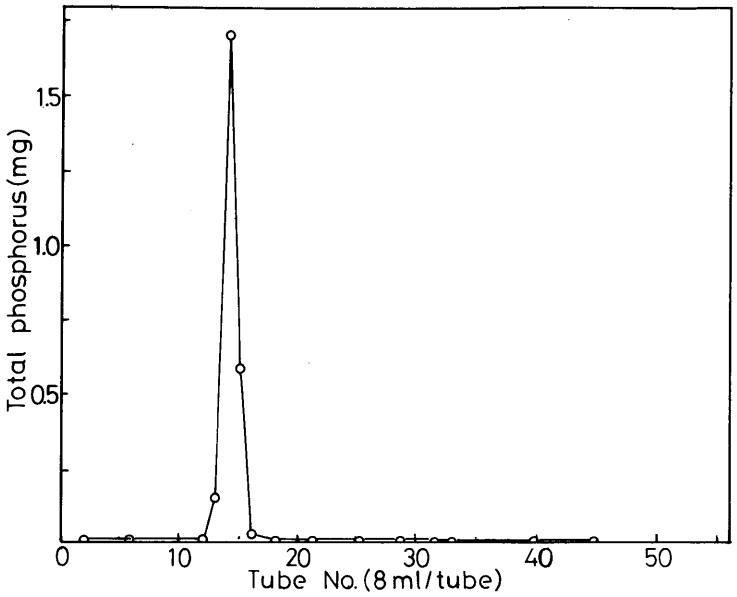

Fig. 4. Rechromatographic elution pattern on a Dowex 50-X 4 column (1 x 50 cm) of the peak (tube No. 24~30) in the Fig. 3.

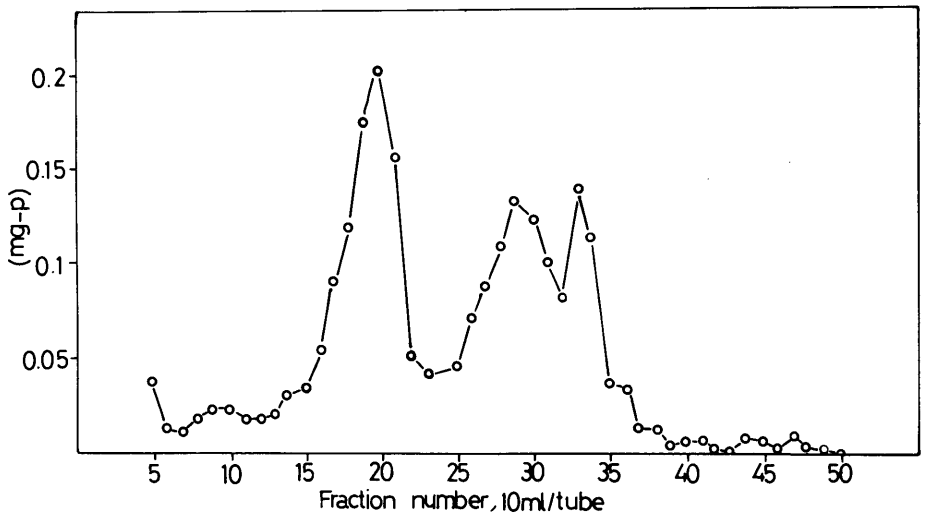

Fig. 5. Elution pattern on a Dowex 50-X 4 column (2 x 30 cm) of organophosporus in the acid hydrolysate of the lipid from GAZAMI, *Portunus trituberculatus*.

trituberculatus.

The tube No. 15~23 and 25~35 inclusive contained ninhydrine- and Rosenberg reagent-positive material on thin layer chromatogram. The fraction between tube No. 15 and 23 of the eluate was rich in phosphorus, and the phosphorus contain-

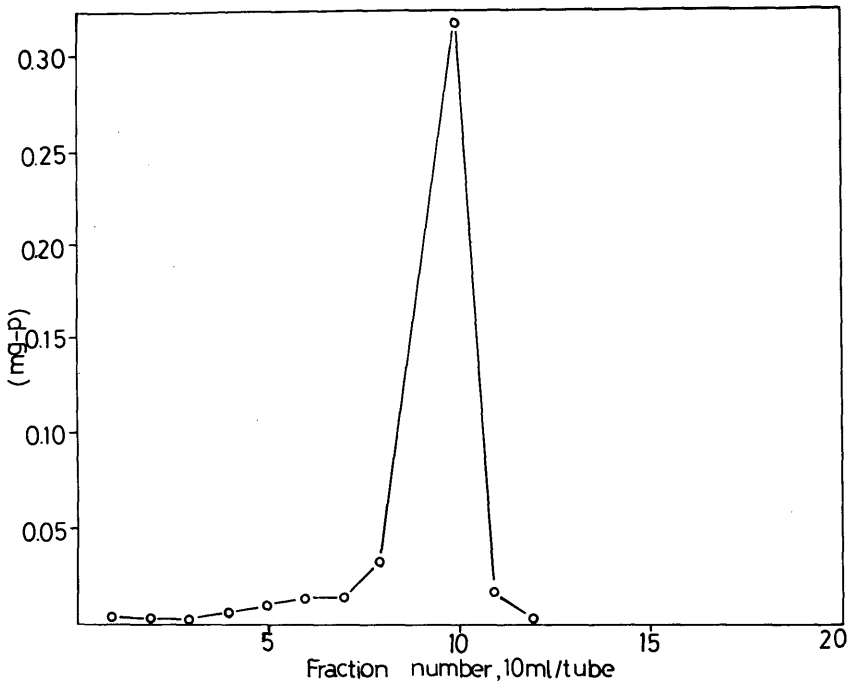

Fig. 6. Rechromatographic elution pattern on a Dowex 50-X 4 column (1.2 x 27 cm) of the peak (tube No. 15~23) in the Fig. 5.

ing substance were found to consist predominantly 2-AEP by thin layer chromatography. This crude material of tube No. 15~23 in the Fig. 5 was further purified on a Dowex 50-X 4 column (1.2 x 27) with 0.6N HCl (Fig. 6), still contained a small amount of contaminants, and was therefore purified by a third passage through the Dowex 1-X 8 column with 0.5N acetic acid. After the removal of acetic acid the residue was dissolved in 0.5 ml of water, and ethanol was added to 50 % concentration. Recrystallization three times from the same solvent yielded 4.5 mg of rhombic crystals which melted with decomposition at 280~281°C.

Anal. Found : C, 19.1 ; H, 6.2 ; N, 11.6 ; P, 25.0.

Calcd. for $C_2H_8NPO_3$: C, 19.2 ; H, 6.4 ; N, 11.2 ; P, 24.8.

Fig. 7 shows the elution pattern on a Dowex 50-X 4 column (2 x 30 cm) of the organophosphorus in the acid hydrolysate of the lipid from UNI, *Hygrosoma hoplacantha*.

The tube No. 25~32, 34~39 and 44~46 inclusive contained ninhydrine- and Rosenberg reagent-positive material on thin layer chromatogram. The fraction between tube No. 25 and 32 of the eluate was rich in phosphorus, and the phosphorus containing substance were found to consist predominantly 2-AEP by thin layer chromatography.

This crude material of tube No. 25~32 in the Fig. 7 was further purified on a

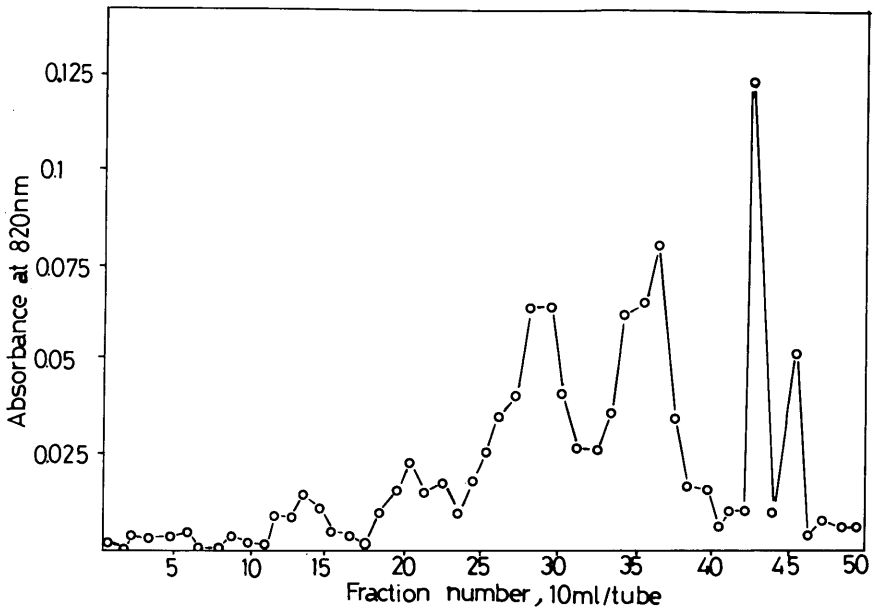

Fig. 7. Elution pattern on a Dowex 50-X 4 column (2 X 30 cm) of organophosphorus in the acid hydrolysate of the lipid from UNI, *Hygrosoma hoplacantha*.

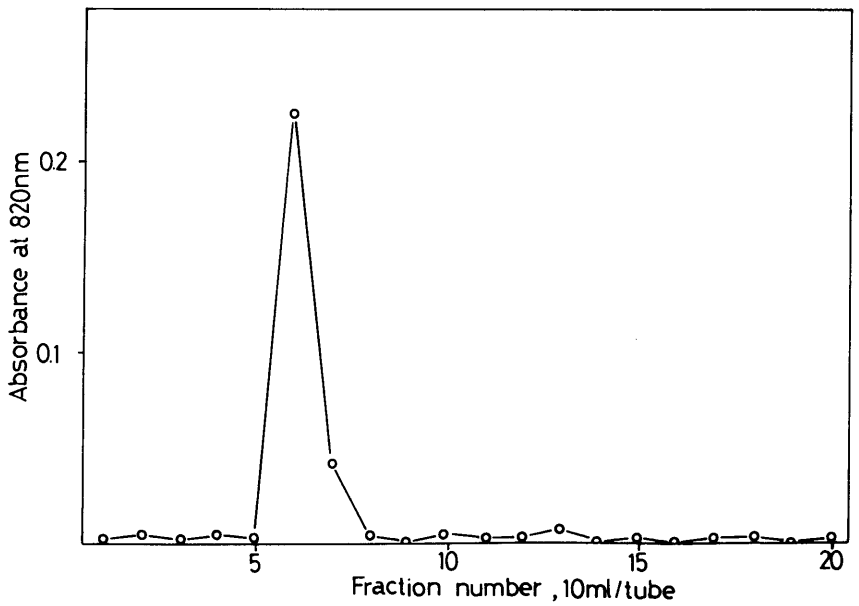

Fig. 8. Rechromatographic elution pattern on a Dowex 50-X 4 column (1.2 X 24 cm) of the peak (tube No. 25~32) in the Fig. 7.

Dowex 50-X 4 column (1.2 X 24 cm) with 0.6N HCl (Fig. 8), still contained a small amount of contaminants, and was therefore purified by a third passage through the Dowex 1-X 8 column with 0.5N acetic acid.

After the removal of acetic acid the residue was dissolved in 0.5 ml of water, and ethanol was added to 50 % concentration. Recrystallization three times from the same solvent yielded 1.4 mg of rhombic crystals which melted with decomposition at 280~281°C.

Anal. Found : C, 19.5 ; H, 6.3 ; N, 11.4 ; P, 25.1.

Calcd. for $C_2H_8NPO_3$: C, 19.2 ; H, 6.4 ; N, 11.2 ; P, 24.8.

3. *Identification of the isolated compound as 2-AEP*

Upon hydrolysis of a portion of the purified substance with 6N HCl at 120°C for 48 hrs. in a sealed tube, neither change in the chromatographic behaviors of this substance nor liberation of inorganic phosphate was observed.

Phosphorus in the obtained material was detected by the wet ashing. The stability of the compound to acid hydrolysis suggested the presence of a C-P bond.

On comparison with synthetic 2-AEP, the isolated compound behaved like 2-aminoethylphosphonic acid in thin layer chromatography with five different solvent systems (Table 2).

Table 2. R_f values on thin layer chromatography of authentic 2-AEP and isolated compound.

Compound \ Solvent	1	2	3	4	5
Authentic 2-AEP	0.58	0.37	0.12	0.12	0.13
from Tengunishi	0.58	0.37	0.11	0.12	0.13
from Uchiwaebi	0.60	0.38	0.11	0.13	0.12
from Gazami	0.59	0.37	0.11	0.12	0.13
from Uni	0.58	0.38	0.11	0.12	0.12

On comparison with synthetic 2-AEP, the melting points of the isolated material and the synthetic product were identical and there was no depression of the mixed melting point (280-281°C). The infrared spectra were virtually identical (Fig. 9). The spectrum showed an absorption band at 1180 cm^{-1} due to C-P bond, and was essentially identical with that of β-form crystal of 2-AEP.

Two crystalline form of 2-AEP have been reported by Horiguchi and Kandatsu [9] ; α-form being rhombic or plate like and β-form being needle. According to their report, the elementary analysis, the chemical properties, and the chromatographic behaviors of both crystallines were identical ; but an infrared spectrum of α-crystal differed from that of β-crystal, and a α-β transition was also demonstrated under some conditions. The infrared spectrum of the substance crystal-

lized initially showed that it was a mixture of both crystalline forms. After recrystallization the infrared spectrum of the isolated material was identical with that of the β-crystal.

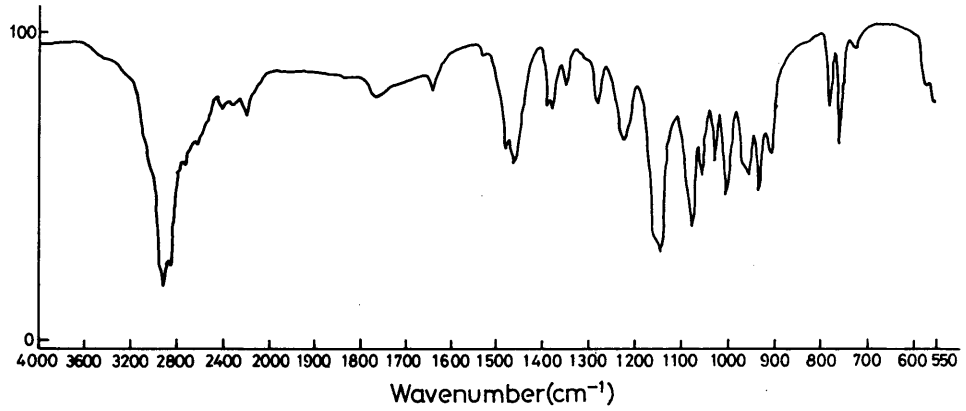

Fig. 9. Infrared spectrum of 2-AEP isolated from the acid hydrolysate of the lipid of TENGUNISHI, UCHIWAEBI, GAZAMI and UNI with a combination of ion exchange chromatographic techniques (Nujol mull).

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