IDENTIFICATION OF THE UNUSUAL POLYAMINES 3,3'-DIAMINODIPROPYLAMINE AND N,N'-BIS(3-AMINOPROPYL)-1,3-PROPANEDIAMINE IN THE WHITE SHRIMP *PENAEUS SETIFERUS*[1]

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SUMMARY: Polyamines of the white shrimp *Penaeus setiferus* were identified by gas chromatography-mass spectrometry as spermidine, 3,3'-diaminodipropylamine, and N,N'-bis(3-aminopropyl)-1,3-propanediamine. Two diamines were also found but were not identified.

The white shrimp *Penaeus setiferus* inhabits the Atlantic waters along the Southeastern coast of the United States. Consumed in the U.S. at an estimated rate of 500 tons a day (1), it is an economically important marine organism. Analysis of *P. setiferus* polyamines by gas chromatography-mass spectrometry clearly showed that spermidine, two unusual polyamines 3,3'-diaminodipropylamine (DAD)[2] and N,N'-bis(3-aminopropyl)-1,3-propanediamine (BAP) and two unidentified diamines were present.

METHODS AND MATERIALS

Live *P. setiferus* were harvested with a cast net during September, 1974, from the Intracoastal waterway adjacent to the Isle of Palms, South Carolina. Specimens used in this study were 10-15 cm in length, excluding the antennas. The live shrimp were immediately dropped into a Dewar flask containing liquid nitrogen and were transported to the laboratory. The frozen shrimp (10-15 g) were homogenized in 5 volumes (v/w) of 0.1N $HClO_4$ with a Waring blendor followed by filtering through Whatman 2V folded filter paper. The polyamines were extracted from the filtrate with 2 volumes of n-butanol after rendering the solution strongly basic by the addition of solid NaOH. The n-butanol was extracted with one volume of 6N HCl, and the HCl extract was evaporated to dryness with a stream of nitrogen. The polyamines were converted to their trifluoroacetyl (TFA) derivatives by heating them in 200 µl of trifluoroacetic anhydride and 10 mg anhydrous sodium car-

[1] This work was presented in part at the 1977 FASEB meeting (Stillway, L.W. and Walle, T. (1977) Fed. Proc., 36, 652).

[2] Abbreviations: DAD-3,3'-diaminodipropylamine
BAP- N,N'-bis(3-aminopropyl)-1,3-propanediamine

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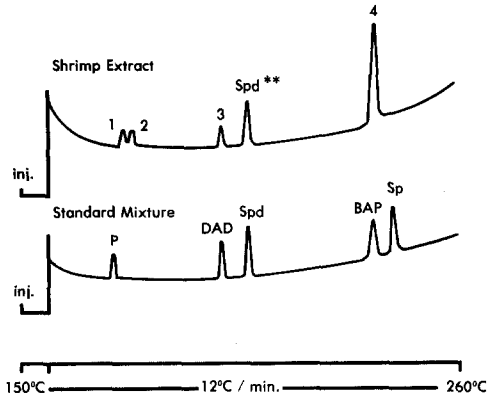

* Analyses were performed on a 2mm x 1.5m glass column packed with 8% OV - 17 coated on silanized 80 / 100 mesh Chromosorb W.

** Abbreviations: Spd - Spermidine
Sp - Spermine
P - Putrescine
DAD - 3,3' - Diaminodipropylamine
BAP - N,N' - Bis (3 - aminopropyl) - 1,3 - propanediamine

FIGURE 1

Gas Chromatograms* of Trifluoracetyl Derivatives of Polyamines Isolated from the White Shrimp *Penaeus setiferus* and a Standard of Polyamines

bonate at 60°C in sealed tubes for 30 min. After cooling 200 µl benzene and 1 ml 1N sodium carbonate were added. Following centrifugation the benzene layer was analyzed for TFA polyamines by flame ionization gas chromatography and by gas chromatography-mass spectrometry. Both analyses were performed with 2mm x 1.5m glass columns packed with 8% OV-17 on silanized 80/100 mesh Chromosorb W. The temperature in each case was programmed 150-260°C at 12°/min. Analysis by gas chromatography was performed on a Varian 1400 gas chromatograph with a nitrogen carrier gas flow rate of 30 ml/min. Analysis by GC-MS was performed on an LKB-9000s using an ionizing voltage of 20 eV and a helium carrier gas flow rate of 20 ml/min as earlier described (2).

Reagent grade $HClO_4$, n-butanol, HCl, benzene, NaOH, Na_2CO_3 and trifluoroacetic anhydride were purchased from Fisher Scientific Co., Atlanta, GA. The benzene was distilled in glass before use. Standard 3,3'-diaminodipropylamine and N,N'-bis (3-aminopropyl)-1,3-propanediamine were purchased from Eastman Organic Chemicals, Rochester, N.Y.

RESULTS

Initially, analysis of the trifluoroacetyl (TFA) derivatives of *P. setiferus* polyamines by gas chromatography showed that according to relative retention times spermidine and several unidentified di- and polyamine species were present as shown in Figure 1. Analysis by GC-MS showed that the

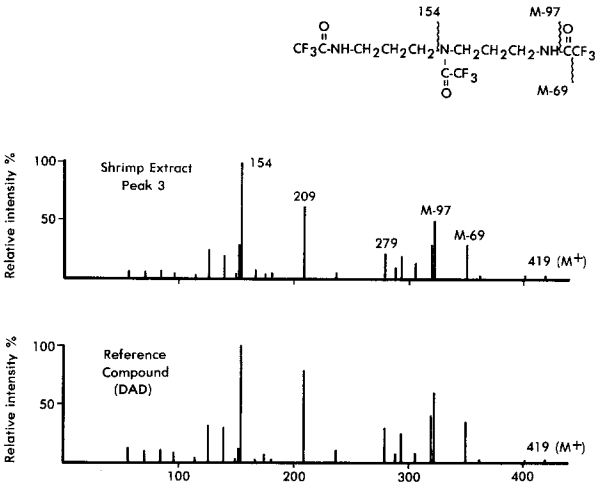

FIGURE 2

Mass Spectra of Trifluoracetyl Derivatives of Peak 3 and Standard 3,3'-Diaminodipropylamine (DAD)

component (peak 1) exhibiting a similar retention time as di-TFA-putrescine did not display a mass spectrum corresponding to standard di-TFA-putrescine, and neither peak 1 nor 2 could be identified. Peak 3 displayed the mass spectrum shown in Figure 2, which was indicative of the tri-TFA-spermidine analog 3,3'-diaminodipropylamine as indicated by the molecular ion at m/e 419 and the fragmentation pattern. The mass spectrum was identical to standard tri-TFA-3,3'-diaminodipropylamine as shown. The next peak yielded an identical mass spectrum as that obtained from tri-TFA-spermidine (2). Peak 4 displayed a mass spectrum (Figure 3) with a molecular ion of m/e 572, indicating that it was the tetra-TFA-spermine analog. As shown, the mass spectrum was identical to that obtained from standard tetra-TFA-N,N'-bis(3-aminopropyl)-1,3-propane-diamine.

DISCUSSION

Both 3,3'-diaminodipropylamine (DAD) and N,N'-bis (3-aminopropyl)-1,3-propanediamine (BAP) were reported for the first time in biological matter only recently (3,4). The presence of DAD had been indicated in plant viruses

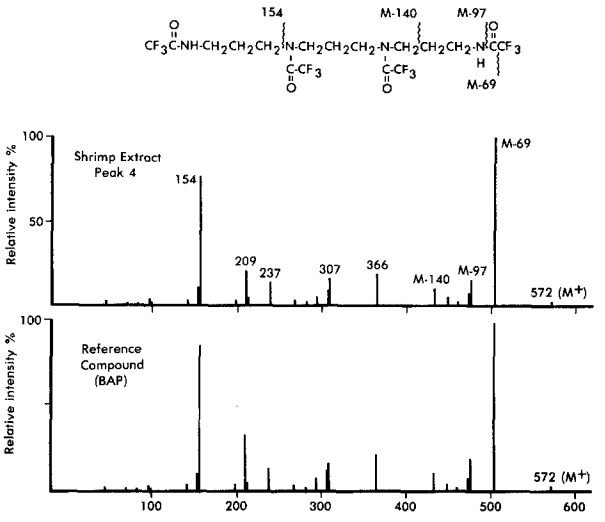

FIGURE 3

Mass Spectra of the Trifluoroacetyl Derivatives of Peak 4 and Standard N,N'-Bis(3-aminopropyl)-1,3-propanediamine (BAP)

in 1962 (5), but later studies could not confirm the presence of this polyamine from this source (6). DAD and BAP have been reported only in certain unicellular thermophiles, and these unusual polyamines have been implicated as necessary components of thermophily (3,4).

Our study clearly shows that both DAD and BAP are present in the multicellular marine organism *P. setiferus*, which is not a thermophile. This finding demonstrates that DAD and BAP are not peculiar to thermophilic organisms, and it leaves the metabolic functions of these unusual polyamines open to speculation. *P. setiferus* did not contain detectable amounts of spermine, and the major polyamine was BAP as shown in Figure 1. A similar pattern was observed by DeRosa et al. (4) in the thermophile *Caldariella acidophila*. DAD and BAP may possess common metabolic roles in the shrimp *P. setiferus* and in the thermophilic bacteria so far studied; but if so, these metabolic functions are probably not related to thermophily.

The only known mechanism by which DAD and BAP may be biosynthesized

is through the metabolic conversion of spermidine to 1,3-diaminopropane followed by transfer of aminopropane units from S-(5'-adenosyl)-3-methyl-mercaptopropylamine (7). Failure to detect 1,3-diaminopropane and putrescine in our study may indicate that in shrimp the flux of carbons through these intermediates is very rapid, or that DAD and BAP are selectively obtained from the diet.

ACKNOWLEDGEMENTS

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