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SCIENTIFIC NOTE

AUTOFLUORESCENT SUBSTANCES IN SHRIMP[1]

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A recent public controversy in Taiwan, R. O. C. dealt with the occurrence of fluorescent substances regarded as putative additives in commercial dried shrimp. Accordingly, we initiated an investigation of this fluorescence.

A data base survey revealed little in the literature to guide us. Pteridines, which are strongly fluorescent, have been reported in shrimp[4], and two unusual and fluorescent amino acids are known to occur in crustacean cuticle[5,6]. In order to clarify the situation, fresh, frozen and dried shrimp were examined and compared.

MATERIALS AND METHODS

Fresh and frozen shrimp (*Aristaeomorpha*) were obtained from fishermen at Keelung. Commercial dried shrimp were purchased from stores in Taipei, Keelung and Tungkang. They included two species of *Aristaeomorpha* and some euphausiids.

Material consisting of whole shrimp, cuticle, eyes and muscle were severally extracted in $CHCl_3$ or 1 M NH_4OH. Extracts were chromatographed on paper (Whatman No. 1) against reference standards.

Extraction of cuticle. Cuticle, carapace and legs of *Aristaeomorpha* were removed, extracted 3× with methanol, 1× with acetone, 1× with diethyl ether and 1× with methanol. The material was then extracted 3× with 0.5 ml 1 M NH_4OH, extracts were pooled and centrifuged at 3000 rpm 10 min. The supernatant was recovered, chromatographed on paper and on a column of Sephadex G-25 equilibrated with phosphate buffer pH 6.5. A fluorescent band was eluted with 0.5 M NH_4OH, applied to a column of Sephadex G-10, and the fluorescent band eluted again with 0.5 M NH_4OH. Ultraviolet/visible and fluorescence spectra were obtained.

Solvent systems used for paper chromatography were *n*-butanol-acetic acid-water 3:1:1; propanol-10% ammonia 2:1; 80% formic acid-methanol-concentrated HCl 80:15:0.5; methanol-pyridine-morpholine-water 10:40:40:70, and 3% NH_4Cl.

Reference standards were isoxanthopterin, xanthopterin, xanthommatin from eyes of *Drosophila melanogaster*, tyrosine, tryptophan, riboflavin (alkaline solution exposed to sunlight), 4, 4′-disulphonic acid Na salt. The last two substances are reported to have been added to marine products and are strongly fluorescent.

RESULTS

The cuticle of dried and fresh shrimp was

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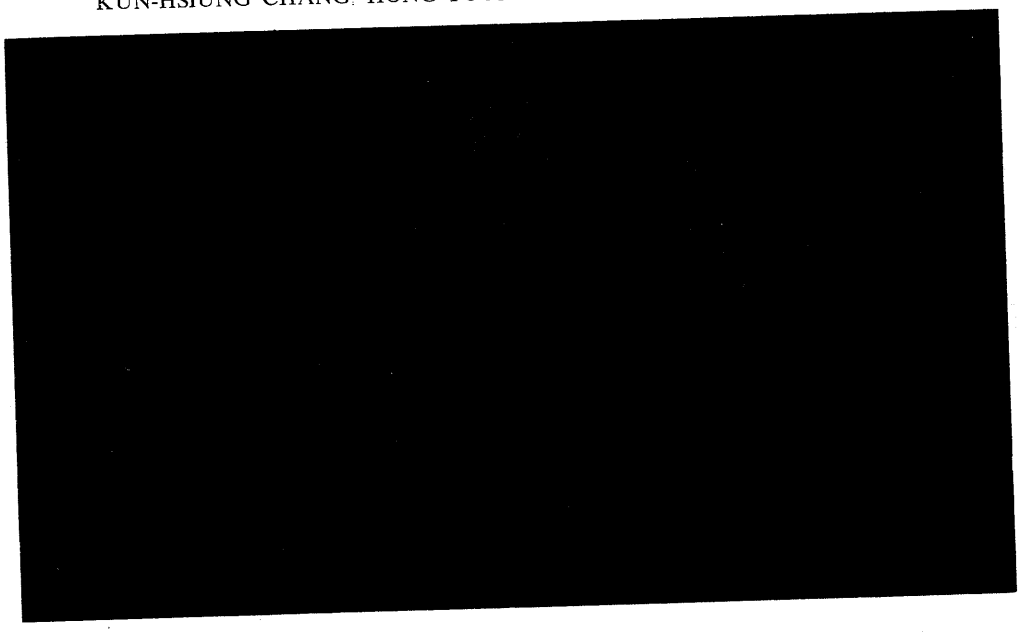

Fig. 1. Autofluorescence from the exterior of the shrimp *Aristaeomorpha* excitated by longwave uv.

blue fluorescent under longwave ultraviolet light (366 nm) (Fig. 1). Close inspection showed that the lateral cuticle, the dorsal cuticle of the abdomen, and the carapace were generally blue fluorescent, whereas the ventral region, including the legs, and the joints of the cuticle were light yellow fluorescent. The blue fluorescent material was water soluble and was readily transferred to filter paper. We also observed blue fluorescence in the cuticle of *Penaeus monodon.*

On chromatograms of extracts of dried and frozen shrimp up to eleven fluorescent spots, some very faint, were seen. There was very little fluorescent material in muscle brei. Extracts of eyes contained several fluorescent fractions (Figs. 2, 3, 4) of which one, conspicuously blue-purple fluorescent, corresponded to isoxanthopterin in fluorescence and colour and colour and Rf value.

The most prominent fluorescent fractions were found in ammonia extracts of cuticle. Extracts were visibly yellow and blue fluorescent (longwave uv, 366 nm). The yellow and blue fluorescent material moved together on Sephadex column. On paper chromatograms there two or three fluorescent factions and a quenching spot (Fig. 3). One fraction was bright purple fluorescent, and differed slightly from isoxanthopterin in fluorescence colour (blue-purple vs purple) and Rf value (slightly higher).

On the basis of chromatographic evidence it was concluded that the detectable fluorescent substances in dried and frozen shrimp were generally similar, and the former contained no fluorescent additive, although most samples of dried shrimp contained a red colouring matter. The drying process, heat, sunlight, could bring about chemical changes and it was decided to concentrate on frozen *Aristaeomorpha.*

Fluorescence spectra were obtained from the exterior of whole shrimp (*Aristaeomorpha*). With excitation at 345 nm, emission from the dorsal cuticle was maximal at 405 nm (shoulder at 423 nm). When fluorescence was observed at 477 nm, the excitation peak was 365 nm. The ventral cuticle had an emission peak at 402 nm and an excitation peak at about 360 nm. With excitation at 285, 310 and 330 nm, emission of the dorsal cuticle was recorded at 400, 420 and 440 nm. A Weber matrix was computed from the results, $\Delta/P = -0.0002$,

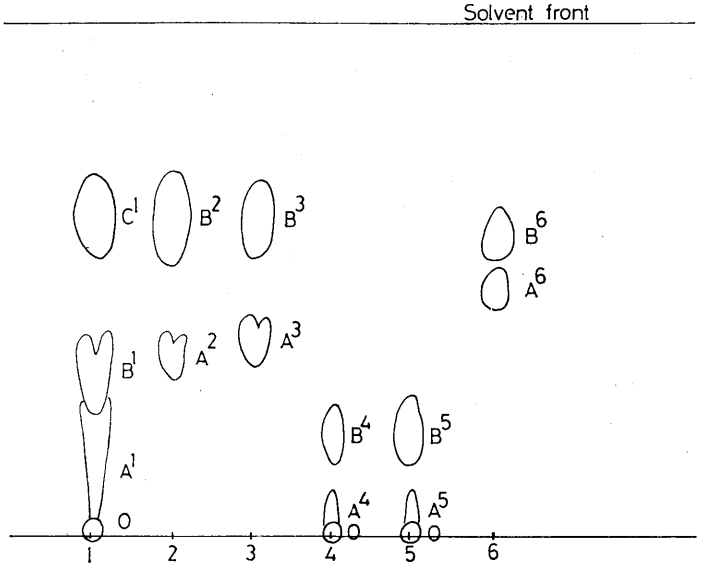

Fig. 2. Paper chromatogram of extracts of *Aristaeomorpha* and reference standards. 1, Eyes. O, origin, reddish. A′, blue-purple streak (ommin?). B^1, blue-purple fluorescent (isoxanthopterin). C^1, blue-grey fluorescent. 2, legs and ventral cuticle. A^2, purple fluorescent. B^2, blue-grey fluorescent. 3, dorsal, lateral cuticle, carapace. A^3, purple fluorescent. B^3, blue-grey fluorescent. 4, 2, 2′-disulphonic acid Na salt. O, A^4, bright blue fluorescent. B^4, blue fluorescent. 5, 4, 4′-diamino-stilbene. Same as 4. 6, xanthopterin. A^6, blue green. B^6, orange. Visualization, longwave uv. Solvent, 3% NH_4Cl.

$3 \times 3 \div 0$, indicating that there were no more than three fluorescent compounds present. A Weber matrix $\Delta/P = 0.125$, $2 \times 2 \div 0$, was indicative one fluorescent emitter[7].

The legs and cuticle and tail region were seperated from the fleshy parts of the body, and fluorescence was recorded from them. Excitation and emission spectra (Fig. 4) were:

	Excitation	Emission
Tail	345 nm	
Legs, ventral region	337 nm	406 nm
Trunk cuticle	345 nm	400 nm

Chromatography on Sephadex G-10 partially resolved two fractions, fluorescing under long- and shortwave uv respectively. Paper chromatograms (Fig. 3) showed two or three fluorescent and a quenching spot and up to four fractions that were ninhydrin positive. One of these had about the same Rf value as tyrosine. A purple fluorescent fraction (A^5, A^6, Fig. 3) was most prominent in the second eluate (II) from Sephadex G-10; a grey-green one was more prominent in eluate I. These two fractions were ninhydrin negative. Other particulars are shown in Fig. 3.

Uv spectra (Fig. 6) were:

Eluate II	250		330 (shoulder)
Eluate from paper	260 (shoulder)	285	333

The fluorescence of eluate II was maximal at λ 426 nm; the excitation curve was maximal at 345 nm. With repeated exposures the emission decreased (Fig. 7). There was another

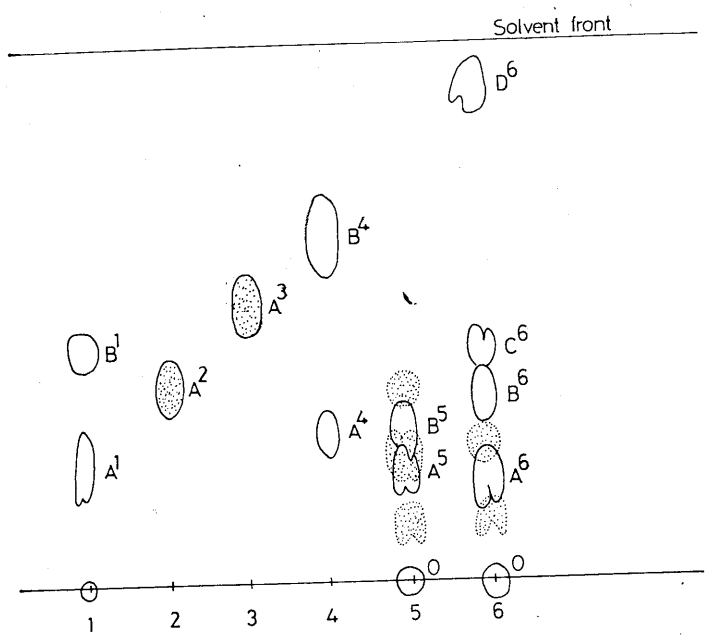

Fig. 3. Paper chromatogram of extracts of the cuticle of *Aristaeomorpha*. Two eluates from a column of Sephadex G-10, and standards. 1, A^1, extract of eyes of *Penaeus monodon*, purple fluorescent, isoxanthopterin. B^1, blue-green. 2, tyrosine. 3, tryptophan. 4, photolyzed riboflavin (alkaline), A^4, yellow. B^4, blue fluorescent. 5, first eluate, visibly yellow, blue fluorescent; O, blue-grey fluorescent (weak); A^5, purple fluorescent; B^5, blue-green fluorescent (shortwave uv). 6, second eluate, blue fluorescent; O, origin, purple fluorescent; B^6, blue-green fluorescent, shortwave uv; C^6, quenching (shortwave uv); D^6, purple fluorescent. Ninhydrin-positive areas stippled. All fluorescence under short uv, unless otherwise indicated. Solvent: *n*-butanol-acetic acid-water, 3:1:1.

lesser emission band centred at 490 nm, with excitation maximal at 400 nm (Fig. 6).

REMARKS

Seemingly similar fluorescent compounds occur in dried and frozen shrimp and, presumably, are natural products. One, blue fluorescent *in situ*, is generally distributed; the other, yellow fluorescent, seems to occur in the legs and at cuticular junctions. They are tentatively correlated with fractions A and B (Fig. 3).

The blue fluorescent material from the cuticle is photolabile (Fig. 7), and its uv spectrum lacks fine detail (Fig. 6). The several fluorescent fractions have not yet been characterized.

The widespread occurrence of fluorescent substance in the shrimp cuticle invites speculation as to their functional significance, which would be better understood if their chemical nature were established. Pteridines, which are fluorescent in their natural state, have been reported for crustaceans[4]. Two unusual and fluorescent amino acids, di- and tri-tyrosine, occur in the cuticle, and are thought to stabilize cuticle protein by the formation of cross-bridges. They have absorption maxima at about 320 nm in alkaline solution. Resilin, an elastic protein

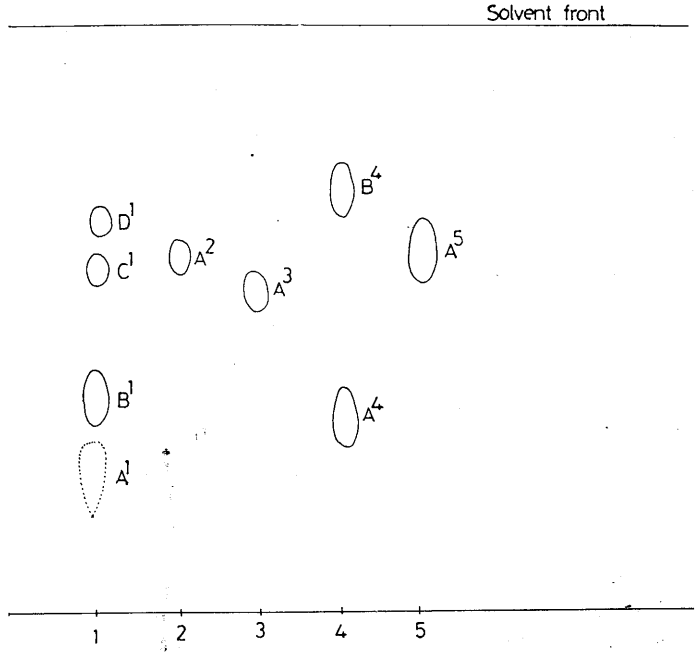

Fig. 4. Paper chromatogram of extracts of the cuticle of *Aristaeomorpha* (0.5 M NH_4OH) and reference materials. 1, eyes of *Penaeus monodon* (0.1 M NaOH). B, blue purple (isoxanthopterin), others weakly fluorescent, grey or grey-purple. 2, eyes of same, 1 M NH_4OH. A^2, purple fluorescent. 3, cuticle, purple fluorescent. 4, eye extract of *Dorsophila melanogaster*. A^4, orange, B^4, blue fluorescent. 5, xanthopterin, A^5, orange fluorescent. Visualization, longwave uv. Solvent system: methanol-pyridine-morpholine-water 10:40:40:70.

formed in joints, which contains these amino acids, has excitation and emission maxima at 253 and 425 nm, respectively[1,2,3,5,6].

These brief observations may be of use in a more detailed investigation of the cuticular fluorescence of shrimp.

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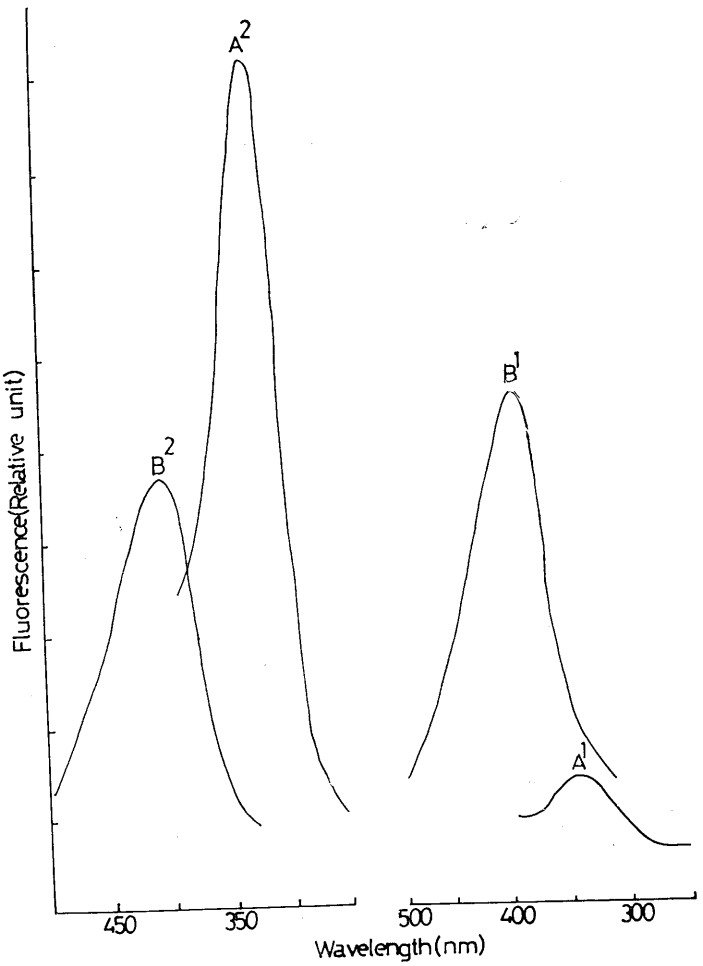

Fig. 5. Fluorescence excitation and emission spectra of *Aristaeomorpha*. The records are from cuticle that had been seperated from the soft parts. A^1, B^1, excitation and emission spectra of trunk cuticle. A^2, B^2, same from legs and ventral region.

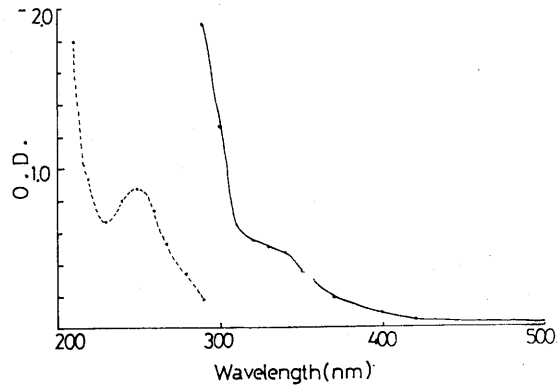

Fig. 6. Absorbance of the blue fluorescent fraction of the cuticle of *Aristaeomorpha* from Sephadex G-10. Solvent 0.1 M NH_4OH. Curve to left, solution diluted $\times 1/10$.

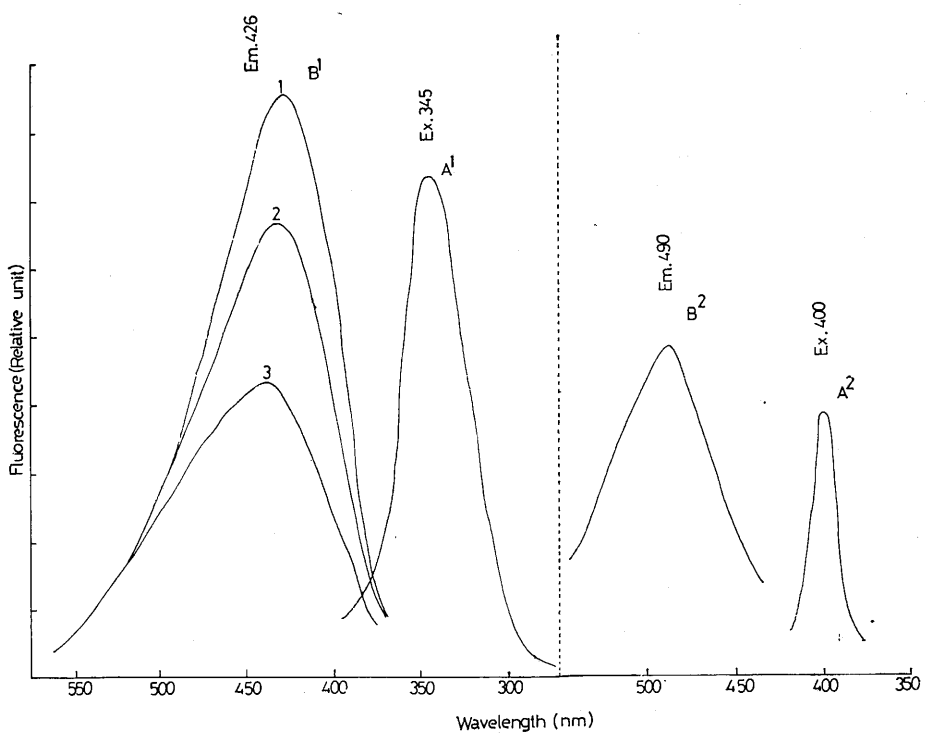

Fig. 7. Fluorescence excitation and emission spectra of material from the cuticle of *Aristaeomorpha*. Eluate from Sephadex G-10 in 0.5 M NH_4OH. A^1, B^1, paired excitation and emission spectra, respectively (1, 2, 3, successive runs). A^2, B^2, second pair of excitation emission spectra, respectively.

蝦米所含天然螢光物質的研究

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本快報係爲瞭解市售蝦米所含天然螢光物質的種類而作。於本研究中，使用鮮蝦、凍蝦及蝦米爲材料。分別對表皮、眼球及肌肉以氨水 (NH_4OH) 及氯仿 ($CHCl_3$) 加以淬取，而後以濾紙色層分析法、薄層板色層分析法及管柱色層分析法，配合不同溶劑系統，加以分離，並使用八種對照標準 (Reference Standards) 化合物以作比較。

綜合各項分析方法所獲得的結果，配合螢光光譜 (Fluorescence spectra) 的分析，並應用 Weber 矩陣的計算，可知蝦米所含天然螢光物質中最主要者爲一種類似異黃蝶呤 (Isoxanthopterin) 的化合物。與鮮蝦、凍蝦所含者並無差異。而且，蝦米並未含有人工螢光添加劑。