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# Isolation and Characterization of a Yellow Pteridine from *Drosophila melanogaster* Mutant *sepia*

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It is well known that the wild type flies of *Drosophila melanogaster* have red and yellow eye-pigments and the mutant *sepia* has only yellow pigments, *i.e.* sepiapterin and isosepiapterin.[1-4] This paper describes the isolation and characterization of an additional pteridine compound from *D. melanogaster sepia*. On the basis of mass spectrum, UV spectra, NMR spectrum and chemical reactions, the structure of the third pteridine compound from the *sepia* flies was proved to be 6-acetyl-2-amino-4-hydroxy-7,8-dihydropteridine.

## Experimental

*Isolation of the Compound (sepiapterin C).*[5-7] *D. melanogaster sepia* was reared in bottles at 25 °C with sterile yeast medium (1000 ml water, 50 g cane sugar, 9 g agar powder, 80 g dry yeast, and 5 ml propionic acid). Flies were harvested on the 1st, 5th, and 9th days after eclosion and stored at −20 °C until extraction.

The flies (50 g) were homogenized in a waring blender with 500 ml of 50% aqueous ethanol for 4 min. The homo-

1) E. Hadorn and H. K. Mitchell, *Proc. Nat. Acad. Sci. U.S.*, **37**, 650 (1951).

2) I. Ziegler and E. Hadorn, *Z. Verebungslehre*, **89**, 235 (1958).

3) H. S. Forrest, C. V. Baalen, and J. Myers, *Arch. Biochem. Biophys.*, **83**, 508 (1959).

4) S. Nawa, This Bulletin, **33**, 1555 (1960).

5) The pteridines from the *sepia* flies are named as sepiapterin A, B, and C systematically, *i.e.*, sepiapterin: sepiapterin A; isosepiapterin: sepiapterin B; actually isosepiapterin is no isomer of sepiapterin.

6) T. Fukushima and M. Akino, *Arch. Biochem. Biophys.*, **128**, 1 (1968).

7) M. Tsusue and M. Akino, *Zool. Mag.* (Tokyo), **74**, 91 (1965).

genate was heated on a boiling water bath for 20 min. It was then centrifuged at 2000 g for 10 min. The extraction procedure was repeated two more times with 50% ethanol (each 400 ml). The supernatants were combined and concentrated to a small bulk at 30 °C. The solution was put on the top of a pH 7 ECTEOLA cellulose column (5×30 cm), and the column washed with distilled water. A yellow, a blue and a purple fluorescent bands were eluted, successively. The eluate of the first band (main band) was evaporated to a small bulk *in vacuo*. The solution was put on the top of a P-cellulose column (4×30 cm), which was then eluted with distilled water. The yellow fraction was separated into three bands; each band was eluted and the eluate evaporated to dryness *in vacuo*. The yellow compounds of the first and the second bands were identified as riboflavin and sepiapterin A, respectively, by means of paper chromatography and UV absorption spectra. The eluate of the third band contained two yellow compounds, which were separated by chromatography on a cellulose column (3×25 cm) using the solvent, 1-butanol, ethanol, water (2:1:1), The substance of the first band was sepiapterin B and that of the second sepiapterin C. The latter fraction was concentrated to dryness and further purified on a Sephadex column (3×28 cm, G-25, fine) (elution: 0.005% ammonia). The eluate was concentrated to dryness and the residue was crystallized from water to give yellow needles, yield, 0.3 mg. The sepiapterin contents (mg/50 g) in sepia flies were calculated from UV absorptions (values on the 1st, 5th, and 9th days after eclosion): sepiapterin A, 19.8, 34.4, 34.4; sepiapterin B, 0.36, 1.33, 1.87; sepiapterin C, 0.27, 0.36, 0.37.

*Structure of Sepiapterin C.* The mass spectrum was determined with a Nihondenshi JEOL-01SG mass spectrometer (a direct inlet system). The ionizing energy was 75 eV and the temperature of the ion source 120—200 °C. The UV spectra were determined using a Hitachi ESP-3 spectrometer and the NMR spectrum using a JEOL-C60 HL spectrometer.

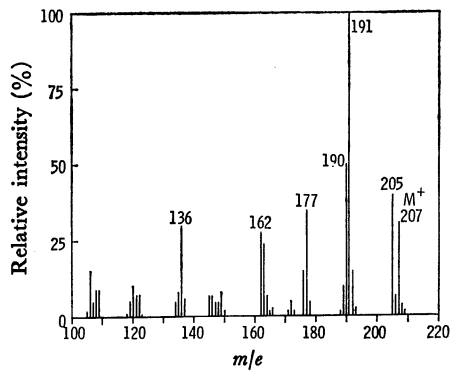


Fig. 1. Mass spectrum of sepiapterin C.

The molecular formula of sepiapterin C was determined to be $C_8H_9N_5O_2$ by mass spectrometry ($M^+$: 207.0774; Calcd for $C_8H_9N_5O_2$, 207.0754) (for mass spectrum, see Fig. 1). Sepiapterin C was proved to be a 6-substituted pterin, as its alkaline permanganate oxidation gives 6-carboxypterin. The formation of 2,4-dinitrophenylhydrazone showed the presence of a carbonyl group at the side chain. The UV-spectra of sepiapterin C are essentially identical with those of sepiapterin A and sepiapterin B (Fig. 2). From these evidences, the structure of 6-acetyl-2-amino-4-hydroxy-7,8-dihydropteridine was postulated for sepiapterin C.

This formulation was well supported by NMR spectrum and the fragmentation pattern of the mass spectrum (the fragmentations are summarized in Fig. 3; the fragment ion,

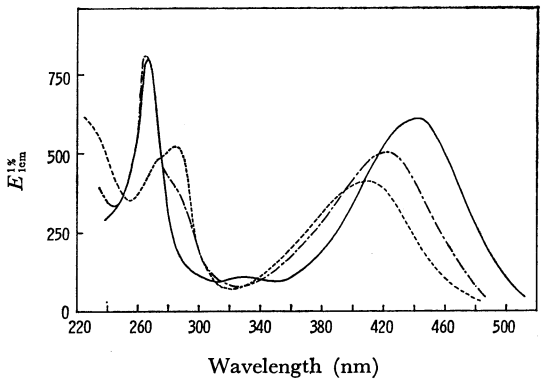


Fig. 2. Ultraviolet absorption spectra of sepiapterin C. in 0.1 M NaOH (——), in $H_2O$ (-·-), and in 0.1 M HCl (-----).

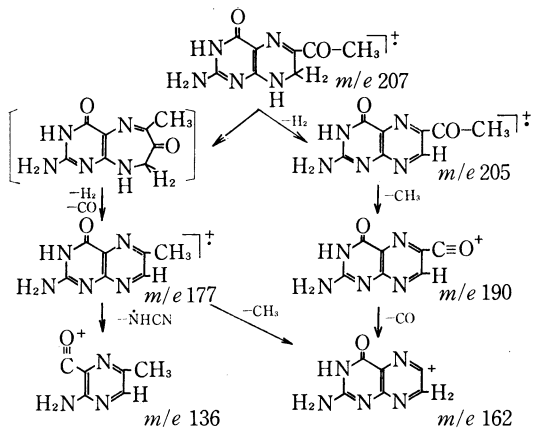


Fig. 3. Fragmentation patterns of mass spectrum of sepiapterin C. The formulae of fragment ions were determined by high resolution mass spectrometry.

Table 1. $R_f$ values of pteridines

| Pteridine | Solvents[a] | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Sepiapterin A | 0.29 | 0.34 | 0.47 | 0.27 | 0.36 |
| Sepiapterin B | 0.21 | 0.44 | 0.52 | 0.46 | 0.53 |
| Sepiapterin C | 0.21 | 0.32 | 0.42 | 0.30 | 0.36 |
| 6-Acetyl-7,8-dihydropterin | 0.21 | 0.32 | 0.42 | 0.30 | 0.36 |

a) Solvents: 1; 3% ammonium chloride, 2; 2-propanol, 1% ammonia (2:1), 3; 2-propanol, 2% ammonium acetate (1:1), 4; 1-butanol, acetic acid, water (4:1:1), 5; 1-propanol, ethyl acetate, water (7:1:2). Ascending method. Whatman filter paper, No. 1.

*m/e* 191 remained to be clarified).[8] NMR (DMSO-$d_6$, 40 °C, TMS): 2.27 ppm (3H, singlet, $-CO-CH_3$), 4.05 (2H, singlet, $-CH_2-$), 6.62 (2H, singlet, $-NH_2$), 7.27 (1H, singlet, -NH-) and 9.96 (1H, singlet, -NH-); the signals of 6.62, 7.27, and 9.96 ppm disappeared with addition of $D_2O$. Finally, 6-acetyl-2-amino-4-hydroxy-7,8-dihydropteridine was prepared by the reaction of 7,8-dihydropterin and pyruvic acid.[9] The identity of the product with sepiapterin C was confirmed by means of paper chromatography, UV and mass spectral determinations. $R_f$-values of pteridines are summarized in Table 1.

8) For mass spectrum of sepiapterin, see; Y. Iwanami and M. Akino, *Tetrahedron Lett.*, **31**, 3219 (1972).

9) H. S. Forrest and S. Nawa, *Nature*, **96**, 169 (1962).