fraction. By contrast, less than 30% of renal postnuclear MUS-sulfatase activity occurred in the microsomal fraction, and about 50% was located in the mitochondrial-lysosomal fraction. Recoveries of all 3 renal sulfatase activities approximated 80±4% of initial postnuclear activity. The unusual distribution of renal MUS-sulfatase activity may be related to differences in the lipid and/or nonpolar amino acid content of the microsomal and non-microsomal enzymes. Only 16±5% of renal MUS-sulfatase activity bound to phenyl-Sepharose, a hydrophobic support, whereas 69±3% of hepatic MUS-sulfatase activity was retained by phenyl-Sepharose. More than 70% of E_1S- and DHEAS-sulfatase activities bound to phenyl-Sepharose, regardless of their tissue of origin. Renal MUS-sulfatase activity displayed a somewhat lower pH optimum, higher apparent K_m, and was less inhibited by E_1S and DHEAS (table 3). These differences are apparently due to enrichment of the hydrophilic MUS-sulfatase isozyme in renal tissue. The phenyl-Sepharose-bound MUS-sulfatase fraction possessed properties approximating those of hepatic MUS-sulfatase activity and was competitively inhibited by DHEAS and E_1S. The phenyl-Sepharose-void fraction of renal MUS-sulfatase had a higher K_m and was not inhibited by E_1S and DHEAS. These trends suggest that only the microsomal (phenyl-Sepharose-bound) MUS-sulfatase is capable of functioning as a steroid sulfatase. The hydrophilic and hydrophobic renal MUS-sulfatase isozymes may be structurally related. This conclusion is supported by their similar thermal denaturation properties, resistance to phosphate and sulfate inhibition, similar inhibition by pNPS (competitive), and the fact that both hydrophilic and hydrophobic MUS-sulfatase activities were increased in SWR/J kidney compared to A/J kidney.

We currently lack evidence for a structural mutation that could account for the SWR/J-A/J interstrain MUS-, E_1S-, and DHEAS-activity variation.

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The occurrence of 7-hydroxybiopterin in the scorpion fly, *Panorpa japonica*

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Summary. A purple fluorescent compound was isolated from the integument of scorpion flies. Based on paper chromatographic, UV-spectrophotometric, fluorometric and HPLC analysis, as well as a chemical color test and various degradation tests, the compound was identified as 7-hydroxybiopterin.

There are many reports of the occurrence of 7-hydroxybiopterin (fig. 1), which has also been named ichthyopterin, in the skin and scales of various fish[2–8]. The chemical structure of the compound was found to be 2-amino-4,7-dihydroxy-6-(1′,2′-dihydroxypropyl) pteridine[9]. Although numerous types of pteridines have been isolated in insects[10–12], 7-hydroxybipoterin has not been reported among them. Two papers discussed the occurrence of a purple fluorescent pteridine in silkworm eggs[13,14]. This compound was named fluorescyanine and it was thought to be identical to or related to 7-hydroxybiopterin. Further studies showed, however, that fluorescyanine in silkworm eggs was isoxanthopterin[15]. In studies on the scorpion fly, *Panorpa japonica*, we found that 7-hydroxybiopterin occurs naturally in this insect's integument. The present paper deals with the isolation and identification of this pteridine from the insect.

Materials and methods. Purified synthetic 7-hydroxybiopterin was kindly supplied by Dr Sugiura of Gakushuin University. Isoxanthopterin and isoxanthopterin-6-carboxylic acid were generous gifts of Prof. Matsuura of Nagoya University. Ethanolyzed cellulose was prepared by the method of Lee and Montgomery[16]. Phospho-Sephadex and Ecteola-cellulose were prepared by the method of Peterson and Sober[17]. All other chemicals were obtained from commercial sources. Scorpion flies were collected at Sagamihara City. The heads and wings were cut from their bodies and the integument was separated from other tissues by pressing between 2 sheets of filter paper.

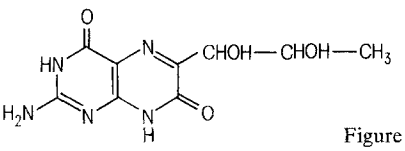

Figure 1. 7-Hydroxybiopterin.

R_f-values, electrophoresis and retention time of HPLC analysis of the related pteridines

Compounds	Paper chromatography solvents 1	2	3	4	5	6	E*	R**
Compound from scorpion flies	0.39	0.29	0.56	0.18	0.61	0.57	4	10.14
7-Hydroxybiopterin	0.39	0.29	0.56	0.18	0.61	0.57	4	10.14
Isoxanthopterin	0.28	0.18	0.42	0.12	0.44	0.32	11	11.93
$KMnO_4$ oxidation product of the compound from scorpion flies	0.18	0.02	0.19	0.0	0.19	0.42	48	-
$KMnO_4$ oxidation product of 7-hydroxybiopterin	0.18	0.02	0.19	0.0	0.19	0.42	48	-
Isoxanthopterin-6-carboxylic acid	0.18	0.02	0.19	0.0	0.19	0.42	48	-

Solvent systems: 1. n-Butanol/acetic acid/water (4:1:2, by vol.). 2. n-Propanol/1% ammonia (2:1, v/v). 3. n-Propanol/1% ammonia (1:1, v/v). 4. n-Propanol/ethyl acetate/water (7:1:2, by vol.). 5. n-Propanol/2% ammonium acetate (1:1, v/v). 6. 3% Ammonium chloride. *Migrated distance (in mm) toward anode after electrophoresis at pH 7.0 (0.02 M potassium phosphate buffer) for 120 min at 34 V/cm. Paper chromatography and electrophoresis were carried out on Toyo No. 51 filter paper. **Retention time (min) of HPLC analysis at 340 nm.

All subsequent steps were carried out in a dark room. About 0.1 g of integument from 11 individuals was cut into small pieces with scissors and homogenized in 10 ml of 50% aqueous ethanol for 4 min. The homogenate was heated in a boiling water bath with a reflux condenser for 20 min. After cooling, the homogenate was centrifuged at $8000 \times g$ for 15 min. The supernatant was concentrated to a small volume by a rotary evaporator. A small amount of Ecteola-cellulose powder (OH form) was mixed into the concentrate and the mixture was then applied to the top of an Ecteola-cellulose column (pH 7.0, 1.5×20 cm). After the column was washed with distilled water, a purple fluorescent compound was eluted with 1% acetic acid. The fluorescent band was detected by UV-light (365 nm). The fraction was collected, concentrated and again applied to the ethanolyzed cellulose column (2.5×30 cm) and developed again with 1% acetic acid. By use of this column 7-hydroxybiopterin was separated from isoxanthopterin, another purple fluorescent compound. The eluated was concentrated and purified by passing through a phospho-Sephadex column (2.0×23 cm) developed with distilled water. The purified substance gave a single purple fluorescent spot on paper chromatography in various solvent systems. Calculating from the molar extinction coefficient[9], the average yield in several extractions was 11 µg per 11 flies. UV-absorption spectra were determined using a Hitachi spectrophotometer 124 type. Fluorescent spectrum was recorded on a Farrand MK-1 spectrophotometer. A model Twincle and a JASCO UVIDEC 100-III were used as the HPLC system. An ion-exchange column of Finepack SIL-NH_2 (4.6×250 mm) was eluted with a solvent system of acetonitrile/water (1:1, v/v). The flow rate was 1 ml/min.

Results and discussion. The UV-absorption spectra of the compound in acid and in alkali correspond exactly with those of authentic 7-hydroxybiopterin (fig. 2). The R_f-values and visible fluorescent colors of the purified compound in various solvent systems also coincided with those of authentic 7-hydroxybiopterin (table). The fluorescent spectrum of the isolated compound was identical with that of authentic 7-hydroxybiopterin using an excitation wavelength of 348 nm (fig. 3). Alkaline potassium permanganate oxidation of the purple fluorescent compound formed isoxanthopterin-6-carboxylic acid as shown by the electrophoretic data in the table. This same compound was formed when synthetic 7-hydroxybiopterin was used in the same test. HPLC analysis of the compound under the experimental conditions shows the same retention time (about 10.14 min) as that of authentic 7-hydroxybiopterin (table). A color test with diazotized sulfanilic acid[18], which is specific for 7-hydroxypterin derivatives, produced orange-red derivatives from both the isolated compound and authentic 7-hydroxybiopterin. Both of the formed derivatives absorbed light at the same peak wavelength. These facts prove that the isolated compound is indeed 7-hydroxybiopterin.

To demonstrate that the isolated compound was not an

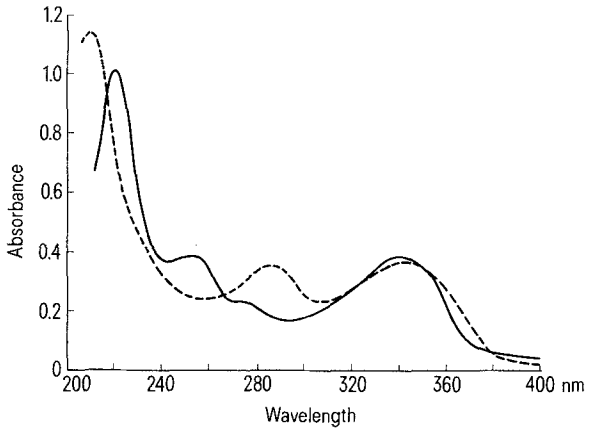

Figure 2. UV-absorption spectra of the purple fluorescent compound isolated from the integument of scorpion flies: (————) in 0.1 M NaOH, (- - - -) in 0.1 M HCl.

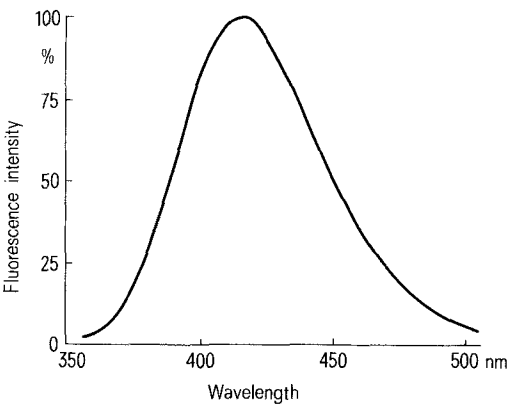

Figure 3. Fluorescent spectrum of the compound in 0.01 M potassium phosphate buffer, pH 7.0. Excitation at 348 nm.

artifact of the acid isolation procedure, a simple alkaline extraction method was next used. A small piece of the intact integument was spotted directly on a thin layer plate and chromatographed with a solvent system of n-propanol/2% ammonium acetate (1:1, v/v). The purple fluorescent substance on the thin layer plate with R_f-value 0.52 was dissolved in 0.1% ammonia water and centrifuged at $3000 \times g$ for 15 min. The supernatant solution was then rechromatographed in various solvent systems. The R_f-values of the compound coincided with those of authentic 7-hydroxybiopterin. This indicates that 7-hydroxybiopterin originates in scorpion fly integument and is not an isolation artifact.

According to Matsumoto[19], 7-hydroxybiopterin is assumed to be a characteristic compound for Cyprinidae. With the report of its occurrence in Salmonidae and with this report of its presence in the scorpion fly, 7-hydroxybiopterin is thus more extensively distributed in nature than previously thought.

Forrest et al.[20] demonstrated that xanthine oxidase/dehydrogenase catalyzes the oxidation of 2-amino-4-hydroxypteridine into isoxanthopterin. However, it was found by means of paper chromatographic analysis that incubations of biopterin and dihydrobiopterin with xanthine oxidase (from cow's milk, Boehringer Mannheim) at pH 7.9 did not result in the formation of either 7-hydroxybiopterin or dihydro-7-hydroxybiopterin. The elucidation of the biosynthetic pathway for this compound and its biological significance in the scorpion fly will be the subject of a future paper.

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Effect of controlled atmospheres on the sorbitol pathway in *Ephestia cautella* (Walker) pupae[1]

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Summary. Exposure of *Ephestia cautella* pupae to hypercarbic atmospheres causes an accumulation of sorbitol in the tissues. This accumulation is maximal at 80% CO_2 and decreases at higher concentrations. The reason for this paradoxical behavior is the inhibition by carbon dioxide of the reduction of glyceraldehyde catalyzed by aldose reductase. This inhibition is competitive and is overcome by accumulation of the substrate. It is suggested that the sorbitol pathway might be a bypass of phosphofructokinase, although its efficiency is questionable.

The enzymes of the sorbitol pathway by which glucose is transformed into fructose via sorbitol have been found in seminal vesicles and placenta of sheep[3,4], in the eye lens[5,6], and in human brain[7]. Various authors have dealt with this pathway in insects: Faulkner[8] found a NADP-dependent polyoldehydrogenase in silkworm hemolymph, Chino[9,10] investigated polyol formation in the silkworm egg during diapause and Kageyama and Ohnishi[11] described the effect of anaerobiosis on the formation of polyols in *Bombyx mori* eggs. This and other stress conditions and their effect on polyol formation in *Callitroga macellaria* eggs have been the subject of investigations by Meyer[12].

We have been investigating the effects of controlled, especially hypoxic and hypercarbic, atmospheres on stored product insects for some time[13,14] in connection with the storage of durable agricultural products under carbon dioxide[15]. The present report deals with studies of these effects on polyol production and the enzymes involved.

Materials and methods. Ephestia cautella pupae were reared according to Navarro and Gonen[16] and treated as described previously[14]. Tissues for metabolite determinations were prepared as described before but were extracted twice with ice-cold perchloric acid (8%), each time at a ratio of 1:5 (tissue weight: acid volume). Glucose and fructose were determined according to Bergmeyer et al.[17] and Bernt and Bergmeyer[18], respectively. Sorbitol was determined according to Bergmeyer et al.[19].

Soluble protein preparations for the determination of enzyme activities were made as follows: Pupae were homogenized in double distilled water (at a ratio of 1:4, w/v) in the