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Accumulation of Inositol by Hibernating Adults of Coccinellid and Chrysomelid Beetles[1]

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Abstract Accumulation of inositol in hibernating adults of some ladybird species, *Henosepilachna pustulosa* (1.9% of body weight), *Eocaria muiri* (1.8%), *Propylea japonica* (1.6%), *Halyzia sedecimguttata* (1.5%), and a chrysomelid species *Paridea angulicollis* (2.3%) is reported. This sugar-alcohol, a substance which has not been reported in hibernating insects, decreased rapidly when these insects ceased their hibernation.

Introduction

Since the first discovery of glycerol and sorbitol accumulated in diapause eggs of *Bombyx mori* (1), the prevalence of glycerol accumulated in hibernating insects has been proved (5). Concomitantly, accumulation of two other substances, trehalose (4) and threitol (2), were also discovered in some insects. Here the occurrence of another sugar-alcohol, inositol, accumulated in hibernating adults of some beetles is preliminarily reported, leaving detailed accounts.

Before going further, I wish to express my sincere thanks to Prof. Shôichi F. Sakagami and Dr. Ichirô Takehara for their reading through the manuscript. Cordial thanks are also due to Dr. Kimio Shimada for his kind advice throughout this study.

Materials and Methods

Halyzia sedecimguttata (Linne) 7 ex., *Henosepilachna pustulosa* (Kôno 5 ex., *Eocaria muiri* Timberlake 1 ex., *Propylea japonica* (Thunberg) 2 ex., and *Paridea angulicollis* Motschulsky 2 ex. were used for analysis. Beetles were collected from the northern slope of Mt. Maruyama, suburb of Sapporo City, northern Japan, in April and May, 1981 from the hibernating sites, i. e. litter layer, except two of *H. pustulosa* which were found on the host plant, thistle. However, some individuals collected in May from the litter were quite active, suggesting cessation of their hibernation. Materials were kept at ca. 5°C in a refrigerator and were subject ot chemical analysis within 24 hours since collecting.

After measuring the body weight, each intact specimen was homogenized

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with 1～2 ml of 80% ethanol containing 0.2 mg of erythritol (internal standard), and centrifuged at 1,500 *g* for 10 min. The obtained supernatant was dried *in vacuo*. To the residue 0.3 ml TRI-SIL 'Z' (Pierce Chemical Co., U. S. A.) was added and heated at 65°C for ca. 45 min. Trimethylsilylated derivatives produced were analyzed by a gas-liquid chromatography (GLC, Shimazu GC-4CMPF) with flame ionization detector, using a glass column (3 m×3 mm i. d.) packed with 1.5% (w/w) OV-1 on Chromosorb W. Column temperature was programmed from 130 to 270°C at 5°C/min and held at 270°C for 7 min. Flow rate of N_2 carrier gas was 40 ml/min.

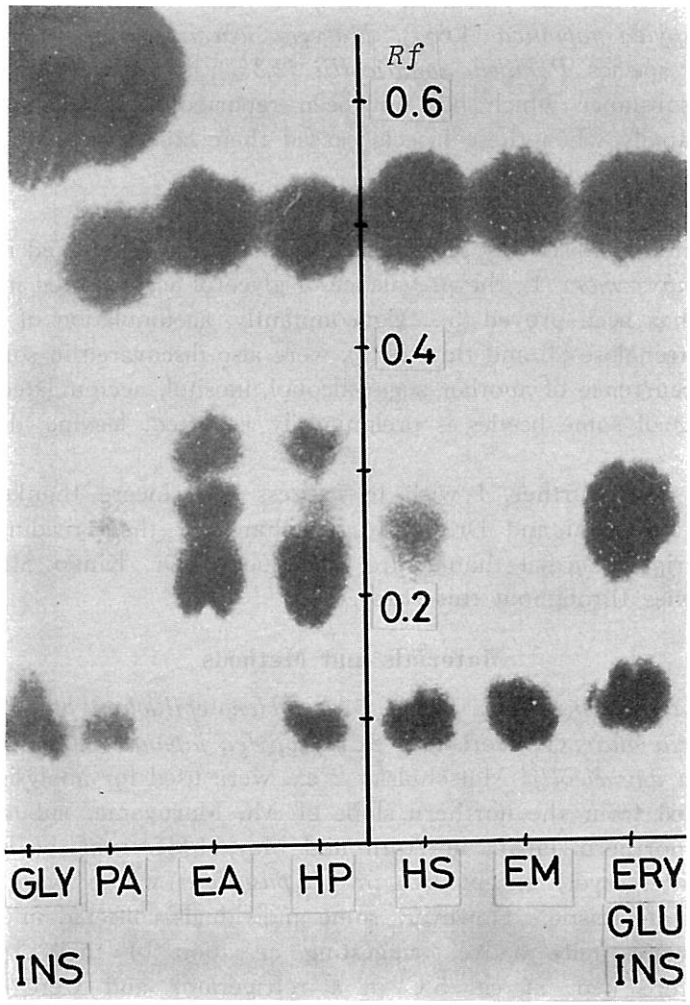

Fig. 1. Paper chromatogram of extracts from *Paridea angulicollis* (PA), *Epilachna admirabilis* (EA), *Henosepilachna pustulosa* (HP), *Halyzia sedecimguttata* (HS) and *Eocaria muiri* (EM) developed with buthanol- acetic acid-water (4 : 1 : 2, v/v), together with some standard reagents, inositol (INS), erythritol (ERY), glucose (GLU) and glycerol (GLY). The scale shows *Rf* value. Erythritol in samples was added artificially for another analysis.

To identify inositol, the supernatant, which was the same sample for GLC, applied to Whatman's chromatography paper No. 1, was developed with *n*-buthanol - acetic acid - water (4 : 1 : 2, v/v) for 16 hours by the ascending method. $AgNO_3$ in alkaline ethanol solution was adopted for the detection of spots.

Results and Discussion

In the course of the analysis an unexpected peak had appeared on gas-liquid chromatogram, of which retention time (t_r) was quite similar to that of inositol standard (t_r=21.6 min). To make sure, a sample to which 0.17 mg of inositol had been artificially added was analyzed. The peak of unknown substance was completely overlapped on that of inositol and exhibited a single enlarged peak. Further, R_f values of the unknown substance and inositol were also quite similar (Fig. 1, R_f=0.10). Thus, the unknown peak can be identified with inositol.

Despite the small sample size and shortness of the period covered, seasonal changes in sugar and sugar-alcohol content analyzed by GLC give certain tendencies (Fig. 2). In *Halyzia sedecimguttata*, inositol occupied 1.5% of body weight in late April and bigan to decrease since early May, while change in trehalose was less conspicuous. Decrease of inositol was more remarkable in *Henosepilachna pustulosa*. In feeding adults collected on May 23, it was detected no more than a trace (0.01~0.02%). Similar

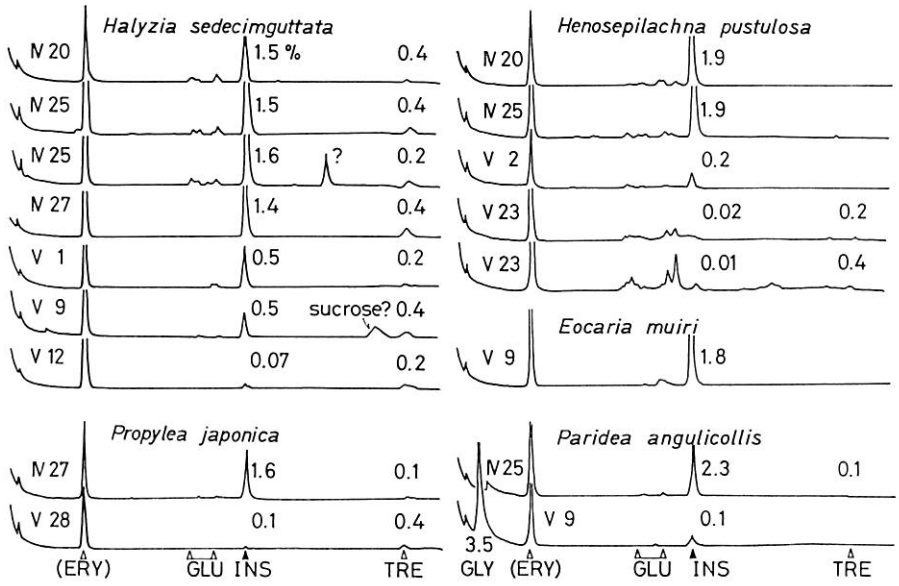

Fig. 2. Gas-liquid chromatogram of trimethylsilyl derivatives prepared from extracts of some beetles. Collected date and percentage (w/w) of the original substance to body weight are given. ERY: erythritol (internal standard), GLY: glycerol, GLU: glucose, INS: inositol, TRE: trehalose

tendencies were also observed in both *Propylea japonica* and *Paridea angulicollis*. Thus, inositol was lost approximately when the beetles ceased the hibernation. It suggests that inositol accumulated in hibernating beetles might contribute to their multi-cryoprotectant system as glycerol (3) or sorbitol and threitol (2) do in other insects.

Curiously, glycerol, the commonest substance accumulated in hibernating insects, was not detected in these beetles, except for *P. angulicollis* collected on May 9. Glycerol may be substituted by inositol in this case. Further information is required to consider their relationships more precisely.

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